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Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions

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Abstract Enteric methane (CH_4) emission in ruminants, which is produced via fermentation of feeds in the rumen and lower digestive tract by methanogenic archaea, represents a loss of 2% to 12% of gross energy of feeds and contributes to global greenhouse effects. Globally, about 80 million tonnes of CH_4 is produced annually from enteric fermentation mainly from ruminants. Therefore, CH_4 mitigation strategies in ruminants have focused to obtain economic as well as environmental benefits. Some mitigation options such as chemical inhibitors, defaunation, and ionophores inhibit methanogenesis directly or indirectly in the rumen, but they have not confirmed consistent effects for practical use. A variety of nutritional amendments such as increasing the amount of grains, inclusion of some leguminous forages containing condensed tannins and ionophore compounds in diets, supplementation of low-quality roughages with protein and readily fermentable carbohydrates, and addition of fats show promise for CH_4 mitigation. These nutritional amendments also increase the efficiency of feed utilization and, therefore, are most likely to be adopted by farmers. Several new potential technologies such as use of plant secondary metabolites, probiotics and propionate enhancers, stimulation of acetogens, immunization, CH_4 oxidation by methylotrophs, and genetic selection of low CH_4-producing animals have emerged to decrease CH_4 production, but these require extensive research before they can be recommended to livestock producers. The use of bacteriocins, bacteriophages, and development of recombinant vaccines targeting archaeal-specific genes and cell surface proteins may be areas worthy of investigation for CH_4 mitigation as well. A combination of different CH_4 mitigation strategies should be adopted in farm levels to substantially decrease methane emission from ruminants. Evidently, comprehensive research is needed to explore proven and reliable CH_4 mitigation technologies that would be practically feasible and economically viable while improving ruminant production.

Keywords Methane production · Ruminants · Mitigation strategies

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Introduction

Greenhouse gas (GHG) emissions have become an increasingly important topic worldwide due to their effects on global warming and climate

change. The effects of GHG emissions on the ecological and socioeconomic vulnerability have already been noticed and will continue to grow regionally and globally in the years to come (IPCC 2007). Carbon dioxide (CO_2), methane (CH_4), nitrous oxide, hydrofluorocarbons, perflourocarbons, and sulfur hexafluoride are the important GHGs that are monitored by the United Nations Framework Convention on Climate Change and have been listed in Annex A of Kyoto Protocol for their mitigation commitment. Global GHG emissions due to human activities (anthropogenic) have grown since the beginning of the industrial revolution with an increase of 70% between 1970 and 2004 (IPCC 2007). Carbon dioxide is the largest contributor of the anthropogenic GHGs representing 76.7% of total anthropogenic GHG emissions in 2004 (IPCC 2007). The global atmospheric concentration of CO_2 has increased from a pre-industrial value of about 280 to 379 ppm in 2005 (IPCC 2007). Methane is the second largest anthropogenic GHG, which contributes 14.3% of total anthropogenic GHG emissions estimated in 2004 (IPCC 2007). The presence of CH_4 in the atmosphere was first discovered in 1948 from features in the infrared absorption spectrum (Migeotte 1948) and is now routinely monitored in the atmosphere. The concentration of CH_4 has increased by about 1,059 ppbv (i.e. from 715 to 1,774 ppbv in 2005) since 1750 (IPCC 2007). Agricultural emissions of CH_4 account for about 60% of the total CH_4 from anthropogenic sources, of which 25% arises from enteric fermentation in livestock (Olivier et al. 2005). Globally, livestock produces about 80 million tonnes of enteric CH_4 annually. Most of the CH_4 from ruminant livestock originates from microbial fermentation of carbohydrates in the rumen and lower digestive tract, referred to as enteric CH_4 emissions. Methane emissions in ruminants also account for a 2% to12% of gross energy loss of feeds depending upon the type of diets (Johnson and Johnson 1995). Therefore, inhibition of CH_4 production in the rumen has been attempted for more than three decades to increase the utilization of feed energy for production purposes. In recent years, CH_4 mitigation research has gained momentum because of the greenhouse effects contributed by CH_4.

The global production of meat, milk, and eggs has increased rapidly during the last decade, particularly in countries with rapid economic development (Steinfeld et al. 2006). The growth of livestock production is expected to continue over the next few decades. This will further stimulate specialization and industrialization of livestock farming and exacerbate GHG problems in the absence of adequate mitigation measures (Steinfeld et al. 2006). Hence, there are urgent needs for development and application of GHG mitigation technologies in livestock production systems. Although a number of reports are available on methane abatement technologies (Moss et al. 2000; Beauchemin et al. 2008; McAllister and Newbold 2008; Eckard et al. 2010), this synthesis discusses several CH_4 mitigation options emphasizing latest developments in this area and identifies future research needs and challenges in the mitigation of enteric CH_4 emissions.

Microbiology of methanogenesis

In 1776, the great physicist Alessandro Volta observed the bubbling of gas in swamps when he was on a boat in his summer holiday. Upon analysis of this gas, he noted that it was flammable and named it as “marsh gas.” After nearly a century, it was confirmed that formation of “marsh gas” (now called CH_4) in these habitats was a microbial process.

In ruminants and pseudo-ruminants like camelidae, the major portion of the methanogenesis occurs in the large fermentative chamber known as rumen, which is located at the beginning of the digestive tract. The rumen is a complex, diverse, and mostly obligate anaerobic microbial ecosystem where feeds including fibrous plant structures are fermented primarily to short-chain volatile fatty acids, CO_2, hydrogen (H_2), and CH_4 by large numbers of different genera and species of bacteria (10^{10} to 10^{12} ml^{-1}), protozoa (10^5 to 10^6 ml^{-1}), fungi (10^4 to 10^5 ml^{-1}), and methanogens (10^8 to 10^{10} ml^{-1}). Methanogens belong to a separate domain archaea in the kingdom of *Euryarchaeota* and are found in a wide range of other anaerobic environments (Liu and Whitman 2008). Most rumen methanogens derive energy for their growth

through a series of biochemical reduction of CO_2 with H_2, and some methanogens use acetate and methyl group-containing compounds to produce CH_4 (methanogenesis).

$$\text{Methanogenesis} : 4H_2 + CO_2 = CH_4 + 2H_2O$$
$$\Delta G^{\circ\prime} = -135.6 \text{ kJ mol}^{-1}$$

Methanogenesis promotes more complete oxidation of fermented substrates and greater energy recovery by fermenting organisms. Besides, it helps to maintain the low partial pressure of H_2 in the rumen, thus providing a favorable environment for degradation of cell wall carbohydrates (Liu and Whitman 2008). Among 28 genera and 113 species of methanogens known to be present in nature, only seven species have commonly been cultured from the rumen (Janssen and Kirs 2008). These are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanoculleus olentangyi*. *Methanosarcina* spp. have also been cultured from the rumen but are not normally a major part of the archaeal community. Analysis of molecular-based studies (Janssen and Kirs 2008) reveals that the members of family *Methanobacteriaceae* (which includes *Methanobrevibacter* spp., *Methanobacterium* spp., and *Methanosphaera* spp.) are the dominant members (30% to 99% of archaea) of the rumen archaea. Members of the order *Methanomicrobiales* (which includes *Methanomicrobium* spp.) are less abundant (0% to 54%), and members of the order *Methanosarcinales* (which includes *Methanimicrococcus*) are rare (2% to 3%). Usually, CH_4 is produced by two types of methanogens, the slow-growing methanogens (generation time about 130 h) that produces CH_4 from acetate (e.g., *Methanosarcina*) and fast growing methanogens (generation time 4–12 h) that reduce CO_2 with H_2. In the rumen, methanogenesis occurs mostly by the fast-growing methanogens as ruminal retention times are too short to permit establishment of the slow growing species (Weimer 1998).

Unlike methanogens, acetogens produce acetate by utilizing H_2. They act as important H_2 sinks in the hindgut fermentation of mammals and termites. Reductive acetogenesis occurs in the intestine of non-ruminants, sometimes along with methanogenesis and sometimes replacing methanogenesis (Liu and Whitman 2008).

$$\text{Acetogenesis} : 4H_2 + 2CO_2 = CH_3COOH + 2H_2O$$
$$\Delta G^{\circ\prime} = -104.6 \text{ kJ mol}^{-1}$$

Acetogens such as *Acetomaculum ruminis* have been isolated from the rumen of most of the domestic species (e.g. Atwood and McSweeney 2008), but population densities of acetogens are highly variable, ranging from non-detectable to 10^5 ml^{-1} rumen fluid (LeVan et al. 1998). Acetogens are the normal flora in the rumen, but methanogens outcompete acetogens as methanogens have lower utilization thresholds for H_2 than acetogens and also due to thermodynamically more favorable nature of methanogenesis over acetogenesis (Atwood and McSweeney 2008).

Methane mitigation options

A schematic diagram of the potential targets of decreasing CH_4 emissions from ruminants has been shown in Fig. 1. Some of the CH_4 mitigation options are confounded with the other possible targets, but they have been included in a particular target considering the main mode of decreasing CH_4 production. For example, ionophores can decrease CH_4 by manipulating rumen fermentation e.g. by inhibiting hydrogen-producing bacteria. They also decrease CH_4 production per unit of products as a consequence of improvement of animal performances. But ionophore compounds have been included as a dietary target because these compounds are generally used as dietary feed additives to increase performance of animals.

Animal inerventions

Number and productivity of animals

Methane production is directly proportional to the number of animals. Culling of nonproductive and low-producing animals is often advocated in developed countries to curtail the CH_4 budget.

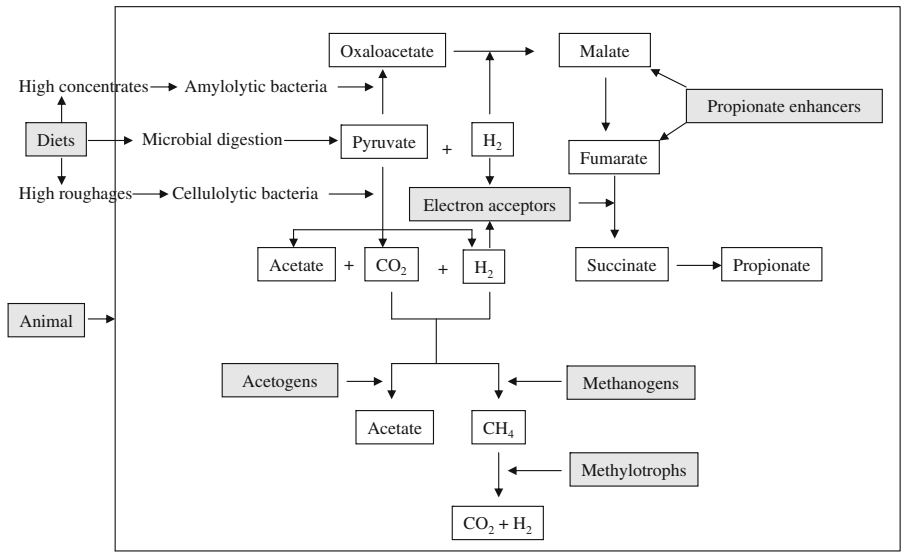

Fig. 1 A schematic presentation of the potential targets of decreasing CH4 emissions from ruminants. *Boxes in dark shade* could be the targets for suppressing CH4 emissions

High-producing animals should be maintained in herds. In this way, although total production will be increased, CH_4 emissions per unit of product could be decreased. This is important so as to supply growing demand of animal products in the years ahead while the impact of emissions could be reduced. However, this is unlikely to be recommended due to socioeconomic and religious background in many developing countries. Proper livestock management especially in developing countries such as reducing the incidence of disease and reproductive problems can decrease CH_4 emission in a herd for each unit of production (Eckard et al. 2010).

Increasing the productivity of animals could also lessen CH_4 emissions per unit of products. There are many options for enhancing the productivity of animals such as supplementation of protein and energy to low-quality forages, ionophores, bovine somatotrophin, probiotics, and proper formulation of diets (Moss et al. 2000). In the Third World countries, genetic potential of animals for production is not expressed due to under or improper nutrition; thus, CH_4 emission could be substantially decreased if a proper feeding management is practiced. It appears that until a proven and reliable CH_4 mitigation technology is developed, minimizing the number of low-producing and unproductive animals and proper feeding practice with increasing the number of high-producing animals could limit CH_4 emission without affecting the total production of animal products.

Genetic selection of animals for decreasing methane emissions

Recently, it has been studied that CH_4 production from different animals under same feeding conditions shows significant variation among animals. In trials with grazing sheep, Pinares-Patiño et al. (2003) identified some animals as high and low CH_4 emitters on the basis of CH_4 output per unit of feed intake and noted that these differences persisted all the four measurement periods of 5 months when the same type of diet was fed. Although the reason is not clear, it might be due to variations of methanogen numbers among animals (Zhou et al. 2009). This finding suggests the possibility of genetic differences between animals in CH_4 production, which could be utilized for genetic selection for low CH_4 production.

Recent research has demonstrated that ruminants with low residual feed intake (RFI; i.e., the difference between actual feed intake and the expected feed requirements for maintenance and production) emit less CH_4 than the animals with high RFI (Alford et al. 2006; Hegarty et al. 2007). This may offer an opportunity for genetic selection for this trait and it can be selected without

compromising the production traits. For instance, Hegarty et al. (2007) reported that CH_4 emission was lower in Angus steers selected based on low RFI than in steers having high RFI (142 vs. 192 g CH_4 day^{-1} or 132 vs. 173 g CH_4 kg^{-1} daily gain) and daily gain was similar in both groups. The low CH_4 emissions by cattle with low RFI might be due to lower methanogen numbers in low RFI cattle than in high RFI cattle (Zhou et al. 2009). It has also been suggested that the greater suppression of CH_4 could be achieved on low digestibility diets, when animals are selected based on low RFI (Hegarty et al. 2007). Thus, this strategy could be more advantageous for the tropical countries where low-quality feeds are fed to ruminants.

Dietary intervention

Ionophore compounds

Ionophore antibiotics such as monensin are usually used in ruminants to improve the efficiency of meat and milk production. They have also been shown to depress CH_4 production in ruminants in dose-dependent manner (Table 1). The CH_4 production has been reported to decrease up to 76% in vitro and to an average of 18% in vivo (Van Nevel and Demeyer 1996). Ionophores do not alter the quantity and diversity of methanogens (Hook et al. 2009), but they change the bacterial population from Gram-positive to Gram-negative organisms with a concomitant change in the fermentation from acetate to propionate. This fermentation shift lowers the availability of H_2 for CH_4 production by methanogens. They might also reduce ruminal protozoal numbers. Relatively high-dose levels might be required to lessen CH_4 compared with doses needed to improve feed efficiency. Monensin included in diets at a dose of <20 mg kg^{-1} diet may not always have profound effect on CH_4 production (Beauchemin et al. 2008). Higher doses (24–35 mg kg^{-1} diet) decreased CH_4 production by 4–10% (Van Vugt et al. 2005; Odongo et al. 2007a) with short-term decreases in CH_4 up to 30% at a dose level of 33 mg kg^{-1} diet (Guan et al. 2006). Unfortunately, some long-term trials suggest that the inhibition of methanogenesis by ionophores may not persist over time (Guan et al. 2006). It appears that monensin can be used for short-term decreases in CH_4 emissions, which can also improve efficiency of feed utilization in ruminants. However, the use of ionophores as feed additives has been banned in the European Union and is restricted in some other countries as feed additives.

Supplementation

In developing countries, low-quality crop residues are fed to ruminants, which are deficient in protein, minerals, and vitamins. Dietary supplementation of these low-quality feeds with energy or protein supplements could reduce CH_4 production as a result of improved efficiency of rumen fermentation. High levels of concentrate feeds in diets increase the propionate production, which decreases H_2 availability for CH_4 production. For example, Lovett et al. (2003) reported that increasing the ratio of concentrate in the diet of beef heifers from 35% to 90% decreased CH_4 production and increased body weight gain. Again, increasing the levels of green fodder such as berseem, oat, and sorghum in straw and stover-based diets may reduce CH_4 release. For instance, methane production in crossbred cows decreased by 33% when green sorghum replaced the wheat straw by 30% (Haque et al. 2001). Similarly, increased feeding of green oat fodder and berseem forage with the wheat straw diets lowered CH_4 production by 8% to 23% and 20% to 30%, respectively, depending on the ratios of green fodders in diets (Singh 2001). The urea-treated straw has also shown to lessen CH_4 emissions in sheep (Sahoo et al. 1999). The use of molasses/urea multinutrient blocks has been found to be a cost-effective diet supplementation strategy with potential to reduce CH_4 emissions by 10% to 25% (Bowman et al. 1992; Srivastava and Garg 2002) and to increase milk production at the same time. Benchaar et al. (2001) evaluated the effect of a range of dietary strategies on CH_4 production using a modeling approach and predicted that CH_4 production could be reduced by increasing concentrate proportions of diets (−40%), replacing fibrous concentrates with starchy concentrates (−22%), with the utilization of less ruminally degradable starch (−7%), increasing the digestibility of forage (−15%), with

Table 1 Effect of monensin on enteric methane production in vivo and animal performance

Animals	Duration (days)	Dosage (mg kg^{-1} diet)	Diet (R/C)	Methane (g kg^{-1} DM intake)[a]		Comments	References
				Control	Monensin		
Sheep	14	10	50:50	23.4a	16.9b	Feed efficiency improved	(Joyner et al. 1979)
Sheep	14	20	50:50	23.4a	15.8b	Feed efficiency improved	(Joyner et al. 1979)
Growing steers	15	48.8	20:80	24.2a	20.4b	–	(Thornton and Owens 1981)
Growing steers	15	37.0	50:50	28.5a	23.8b	–	(Thornton and Owens 1981)
Growing steers	15	37.0	67:37	32.1a	24.5b	–	(Thornton and Owens 1981)
Steers	42	39.7	20:80	18.6a	14.1b	–	(Wedegaertner and Johnson 1983)
Dairy cows	21	24	65:35	29.0a	25.3b	Improved feed efficiency and milk production	(Sauer et al. 1998)
Dairy cows	21(previous exposure)	24	65:35	27.2	23.9	Feed efficiency and milk production unaffected	(Sauer et al. 1998)
Beef cattle	19	33	75:25	22.6	20.7	–	(McGinn et al. 2004)
Dairy cows	11	29.6	Ryegrass	16.9a	15.3b	–	(Van Vugt et al. 2005)[b]
Non-lactating cows	72	35.2	Ryegrass	25.5	24.8	–	(Van Vugt et al. 2005)[b]
Dairy Cows	23	17.5	Ryegrass + white clover	17.5	16.9	–	(Van Vugt et al. 2005)[b]
Dairy cows	58	18.1	Ryegrass + maize silage	19.2	20.5	–	(Van Vugt et al. 2005)[b]
Beef cattle	112	33	86:14	18.8a	13.2b*	No effect on ADG and feed efficiency	(Guan et al. 2006)
Beef cattle	112	33	31:69	15.0a	11.0b**	No effect on ADG, improved feed efficiency	(Guan et al. 2006)
Dairy cows	180	24	60:40	23.3a	22.4b	Milk production unaffected	(Odongo et al. 2007a)
Dairy cows	77	10.8	100:0	19.2	20.0	No effect on milk yield	(Waghorn et al. 2007)[b]
Dairy cows	78	13	72:28	16.7	17.0	Improved efficiency of milk production	(Grainger et al. 2008)[b]
Dairy cows	78	13	72:28	16.7	17.0	Improved efficiency of milk production	(Grainger et al. 2008)[b]

Methane production data followed by small letters in row differ at $P < 0.05$. *DM* dry matter, *R/C* roughage to concentrate ratio, *ADG* average daily body weight gain

[a]When methane values were not presented as g kg^{-1} DM intake, the values were calculated from the reported data

[b]Monensin controlled-release capsules were used, which might perform improperly

*$P < 0.05$ (significant effect up to 42 days, thereafter no effect of monensin), **$P < 0.05$ (significant effect up to 28 days, thereafter no effect of monensin)

legumes compared to grass forages (−28%), and with silages compared to hay (−20%).

Forage species

Some legume forages have been shown to decrease CH_4 production in ruminants, which are often explained by the presence of condensed tannins (CT), low fiber content, high dry matter (DM) intake, and faster rate of passage from the rumen (Table 2; Beauchemin et al. 2008). A decrease in CH_4 production was observed in Rusitec as the proportion of sainfoin (*Onobrychis viciifolia*) increased in the diets (McMahon et al. 1999). Woodward et al. (2002) investigated the feeding of sulla (*Hedysarum coronarium*) on CH_4 emission and milk yield in Friesian and Jersey dairy cows. Cows fed on sulla produced less CH_4 per kg DM intake (19.5 vs. 24.6 g) and per kg milk solid yield (243.3 vs. 327.8 g). Similar trends in CH_4 emission and milk production have been observed in dairy cows fed on birdsfoot trefoil (*Lotus corniculatus*) silage compared with dairy cows fed on ryegrass pasture (Woodward et al. 2001). There was also a 16.7% decrease in CH_4 production per kg DM intake in lambs fed on lotus (*Lotus pedunculatus*) compared in lambs fed on lotus and polyethylene glycol (which inactivates CT by binding with it), which is attributed to the presence of CT in lotus (Waghorn et al. 2002). Animut et al. (2008a) also observed that feeding of different levels of kobe lespedeza (*Lespedeza striata*) decreased CH_4 production linearly in goats, and it has been attributed to the presence of CT (Animut et al. 2008b). Furthermore, it has been reported that C3 forages such as ryegrass and wheat might yield less CH_4 per unit of digestible DM than C4 forages such as corn and sorghum (Ulyatt et al. 2002), presumably due to high content of fiber in C4 plants, but more studies are needed to explain this result.

Suppression of rumen methanogens

Chemical compounds

For a long time, halogenated CH_4 analogs and related compounds such as chloroform and chloral hydrate were tested for CH_4 production inhibition in ruminants. However, they cause liver damage and death of animals after a long period of feeding. Therefore, it appears that they are not suitable for use in practice. Amichloral (a hemiacetal of chloral and starch) decreased CH_4 production and increased live weight gain, but its antimethanogenic activity decreased gradually with prolonged feeding (Trei et al. 1972). Similarly, the effects of trichloroacetamide and trichloroethyl adipate on ruminal methanogenesis were reported to be transient. Bromochloromethane and 2-bromoethanesulfonic acid, a bromine analogue of coenzyme F involved in methyl group transfer during methanogenesis decreased CH_4 outputs (Dong et al. 1999), but their anti-methanogenic activity was reported to be transient; however, a combination of bromochloromethane and α-cyclodextrin was found to be more stable and were capable of suppressing CH_4 emissions in ruminants over a prolonged period (McCrabb et al. 1997). Garcia-Lopez et al. (1996) and Kung et al. (2003) reported that 9,10-anthraquinone inhibited methanogenesis, and it is speculated that 9,10-anthraquinone inhibits the reduction of methyl co-enzyme M to CH_4 by uncoupling electron transfer in methanogens. Recently, iodopropane both in vitro (Mohammed et al. 2004a) and in steers without affecting digestibility (Mohammed et al. 2004b), and diallyl maleate in vitro and in vivo (Lila et al. 2004) have been shown to suppress CH_4 production. There was no apparent adaptation of these compounds to ruminal microbes up to 21 (Lila et al. 2004) and 25 (Mohammed et al. 2004b) days. Feeding of α-cyclodxtrin iodopropane complex (to prevent volatility and pungent odour of iodopropane) to steers for a period of 25 days had no apparent health problems (Mohammed et al. 2004b). Iodopropane is probably a corrinoid inhibitor that transfers methyl group to coenzyme M in methanogens.

Some nitrocompounds such as nitroethane, 2-nitroethanol, 2-nitro-1-propanol, and 3-nitro-1-propionic acid inhibited ruminal CH_4 production in vitro (Anderson et al. 2003, 2008) and nitroethane and 2-nitro-1-propanol have been shown to reduce CH_4-producing activity in vivo (Anderson et al. 2006; Gutierrez-Bañuelos et al. 2007). These nitrocompounds probably act by

Table 2 Effects of forages on in vivo methane production and fermentation in the rumen

Forage species	Animals (duration)	Proportion of forage	Control diet	Methane inhibition[a]	Methane inhibition[b]	Comments	References
Hedysarum coronarium forage (2.72% CT)	Dairy cows (12 days)	*H. coronarium* as sole feed	Ryegrass pasture	2.35%	20.7%[c]	–	Woodward et al. (2002)
H. coronarium forage (6.8% CT)	Sheep (10 days)	*H. coronarium* forage as sole feed	Ryegrass pasture	–	30.5%	Digestibility unaffected	Waghorn et al. (2002)
Lespedeza cuneata (contains 17.7% CT)	Goats (120 days)	*L. cuneata* pasture	Crabgrass/tall fescue pasture	30.2%	50.2%	TVFA and A/P unaffected	Puchala et al. (2005)
Lespedeza striata forage	Goats (21 days)	*Lespedeza:* sorghum-sudan grass (1:2, 2:1 and 3:0)	Sorghum-sudan grass	32.9, 47.3 and 58.4%	29, 38.7 and 74.5%	Digestibility and protozoal numbers decreased, TVFA and A/P unaffected	Animut et al. (2008a)
L. striata forage (15.1% CT)	Goats (36 days)	*L. striata* as sole feed	*L. striata* + polyethylene glycol	49.5%	33.3%	Digestibility and protozoal numbers decreased, TVFA and A/P unaffected	Animut et al. (2008b)
Lotus pedunculatus forage	Sheep (7 days)	*L. corniculatus* as sole feed	Ryegrass pasture	No effect	28.9%[c]	–	Woodward et al. (2001)
L. pedunculatus forage	Sheep (7 days)	*L. corniculatus* as sole feed	Lucern pasture	No effect	23.7%[c]	–	Woodward et al. (2001)
Lotus corniculatus silage (2.59% CT)	Dairy cows (12 days)	*L. corniculatus* as sole feed	Ryegrass silage	No effect (8.57%)	23.4%[c]	–	Woodward et al. (2001)
L. pedunculatus forage (5.3% CT)	Sheep (10 days)	*L. pedunculatus* forage as sole	*L. pedunculatus* forage + PEG	–	5.2%	Digestibility decreased	Waghorn et al. (2002)

TVFA total volatile fatty acids concentration, *A/P* acetate to propionate ratio, *CT* condensed tannins; *PEG* polyethylene glycol to bind tannins

[a]Inhibition of methane production compared with control on volume basis

[b]Inhibition of methane production compared with control relative to dry matter or organic matter digested unless otherwise marked

[c]Relative to per kilogram feed intake

Table 3 Effect of addition of fat in diets on enteric methane production in vivo

References	Animals	Diet (R/C)	Duration (days)	Added fat%	Fat type	Methane production (g kg^{-1} DM intake)[a]		Digestibility	Milk yield or ADG
						Control	Fat[a]		
(Beauchemin et al. 2007)	Beef heifers	65:25	21	3.4	Tallow	20.0a	17.8b	=	=
					Sunflower oil	20.0a	17.7b	=	=
					Sunflower seeds	20.0a	15.4	–	=
(Beauchemin and McGinn 2006)	Beef Heifers	75:25	21	4.6	Canola oil	25.5	21.7	–	=
(Beauchemin et al. 2009)[b]	Dairy cows	45:55	28	4.2	Sunflower seeds	16.3	14.6	–	=
				3.7	Flax seeds	16.3a	13.4b	–	=
				3.9	Canola seeds	16.3a	13.7b	=	=
(Holter et al. 1992)	Dairy cows	100:0	112	2.7	Cottonseed	13.4a	12.1b	–	–
(Jordan et al. 2006a)	Beef heifer	50:50	35	1.33	Coconut oil	28.3	25.9	=	NR
				2.71	Coconut oil	28.3a	24.1b	=	NR
				4.57	Coconut oil	28.3a	21.1b	–	NR
(Jordan et al. 2006b)	Beef heifer	50:50	74	8.0	Coconut oil	27.5a	22.1b	=	+
				7.4	Copra meal	27.5a	23.6b	–	=
(Jordan et al. 2006c)	Beef heifer	10:90	103	6.0	Soybean oil	12.7a	8.0b	NR	+
					Soybean seeds	12.7a	10.8b	NR	=
(Lovett et al. 2003)	Beef heifer	65:35 to 10:90	77	4.65	Coconut oil	22.4a	17.3b	NR	=
(Machmüller et al. 2003a)[b]	Sheep	57:43	15	6.0	Coconut oil	18.3	17.5	NR	NR
(Machmüller et al. 2003b)	Sheep	1:1.5 and 1:0.5	15	5.0	Myristic acid	23.4a	13.7b	=	NR
(Machmüller and Kreuzer 1999)	Sheep	71:29	23	3.5	Coconut oil	24.8a	19.4b	=	NR
		45:54	23	7.0	Coconut oil	24.8a	8.8b	=	NR
(Mao et al. 2010)	Lamb	60:40	60	3.0	Soybean oil	18.6a	16.0b	NR	=
(Martin et al. 2008)	Dairy cows	65:35	28	4.2	Crude linseed	21.1a	18.9b	–	=
				4.4	Extruded linseed	21.1a	15.5b	–	–
				5.8	Linseed oil	21.1a	10.1b	–	–
(McGinn et al. 2004)	Beef cattle	75:25	21	5.7	Sunflower oil	22.6a	18.8b	=	NR
(Odongo et al. 2007b)	Dairy cows	61:39	11	5.0	Myristic acid	28.4a	19.5b	NR	=

Methane production data followed by small letters in row differ at $P < 0.05$. *R/C* forage to concentrate ratio, *ADG* average daily body weight gain, *NR* not reported

[a]When methane values were not presented as grams per kilogram DM intake, the values were calculated from the reported data

[b]Control diet was added with rumen protected fat

inhibiting H_2 and formate oxidation (Anderson et al. 2008). Although these studies on chemical antimethanogenic agents show promise to lower CH_4 emissions, research on these chemical feed additives is unlikely to be continued due to public concerns over chemical residues in products of animal origins. If these compounds are supported for use as antimethanogenic compounds due to noble cause of reducing greenhouse effects, there is need of thorough research for their effects on animal health and presence of these chemicals in animal products along with the withdrawal period of these compounds.

Fat addition

Fat inclusion in the diets causes a decrease in CH_4 production depending upon the levels of fat supplementation, fat sources, forms of fat supplementation, and types of diet (Table 3). Irrespective of fat sources, CH_4 emissions (grams per kilogram of DM intake) were calculated to be reduced by 5.6% with each 1% addition of fats (Beauchemin et al. 2008). A decrease in CH_4 production by fat supplementation may be mediated through combined influences on the inhibition of growth of methanogens and protozoal numbers and reduction of ruminal organic matter (OM) fermentation and hydrogenation of unsaturated fatty acids (acting as a alternative H_2 sink) in the rumen. There are considerable variations in the CH_4 reduction among fat sources, with marked reduction occurring for refined medium chain fatty acids (i.e., C12:0 and C14:0) such as coconut oil (64% at 7% level), myristic oil (58% at 5% level), canola oil, and palm kernel oil compared with C18 fatty acids (Machmüller and Kreuzer 1999; Machmuller et al. 2003a, b). Although fat inclusion in diets lowers CH_4 emissions consistently for long periods, fat particularly at concentrations above 6–7% of dietary DM can significantly diminish DM digestion particularly fiber components and DM intake, and again the severity of the effect varies with the fat used and type of diets (Machmüller et al. 2003b; Beauchemin et al. 2008). Besides, high levels of added fat can reduce milk fat percentage and daily gain or milk yield (Martin et al. 2008). Therefore, care must be taken in choosing the appropriate fat sources and level of fat supplementation. Fat supplementation increases the energy density of diets, which might also improve animal performance (Grainger et al. 2008) and feed efficiency despite reduced feed intake (Jordan et al. 2006b, c). Sometimes, fat supplementation decreases or does not affect the performances of animals; however, it suppresses CH_4 outputs per unit of products. For example, feeding of whole soybean seeds did not affect body weight gain but lowered CH_4 emissions per kilogram gain (Jordan et al. 2006c). Similarly, fat supplementation through extruded linseed decreased milk yield probably due to reduced feed intake and digestibility, but significantly decreased CH_4 emissions per kilogram of milk yield. In contrast, supplementation of fat through whole cottonseed decreased CH_4 (grams per day or gram of per unit of products) and also increased milk production when dairy cows grazed in low quality pastures (Grainger et al. 2008). It appears that proper supplementation of fat is a promising technology for mitigation of CH_4 on consistent basis without affecting production. However, cost of fat supplementation with edible oils might not be economical for the livestock producers.

Plant secondary compounds

Recently, bioactive plant metabolites have been an important area of research to substitute chemical feed additives. Many phytochemicals such as saponins, tannins, essential oils (Table 4), and many other unknown metabolites from a wide range of plant sources show potential for CH_4 mitigation options (Kamra et al. 2008; Patra et al. 2008; Patra and Saxena 2010). These metabolites lessen CH_4 production through a direct effect on methanogens and/or elimination of protozoa, reduction of OM digestion, and modification of fermentation in the rumen (Patra and Saxena 2010).

Saponins There is increasing evidence to suggest that addition of saponins in the diets might diminish CH_4 production, which is likely due to a decrease in protozoal numbers and/or methanogenic archaeal activity. Saponins of *Sapindus saponaria* suppressed CH_4 production by 20% without affecting methanogen numbers in Rusitec (Hess et al. 2003) or in lamb (Hess et al. 2004). Agarwal

Table 4 Effects of phytochemicals on in vivo methane production and fermentation in the rumen

Phytochemicals	Animals (duration)	Dosage	Diet (R/C)	Methane inhibition[a]	Methane inhibition[b]	Comments	References
Saponins							
Lucerne saponins (27.8% saponins)	Sheep (14 days)	0.2 to 0.8 g kg^{-1} $BW^{0.75}$ or 10 to 40 g kg^{-1} diet	100:0	No effect	No effect	Digestibility decreased at 2 and 4% concentrations; TVFA and A/P unaffected;	Klita et al. (1996)
Quillaja saponaria extract (5 to 7% saponins)	Sheep (18 days)	13.5 g kg^{-1} diet or 16.1 g day^{-1}	60:40	16.9% (no effect)	21.7%	TVFA decreased, digestibility, A/P and unaffected	Pen et al. (2007)
Q. saponaria plant (3% saponins)	Cattle (28 days)	10 g kg^{-1} of DM	51:49	No effect	7%	Digestibility, TVFA and A/P unaffected	Holtshausen et al. (2009)
Sapindus saponaria fruits	Sheep (21 days)	5 g kg^{-1} $BW^{0.75}$	49.2 to 56:21	6.5%	7.8%	Digestibility and A/P decreased; TVFA and methanogens numbers increased	Hess et al. (2004)
Saponins	Sheep (15 days)	170 mg day^{-1} or 0.13 g kg^{-1} diet	75:25	15.5%	13.7%	Digestibility unaffected, A/P decreased, TVFA increased	Wang et al. (2009)
Sarsaponin	Sheep (15 days)	0.12 g kg^{-1} diet	70:30	7.1%	6.7%	Digestibility, TVFA and A/P unaffected	Santoso et al. (2004)
Sarsaponin (1.25% saponins)	Sheep (21 days)	0.002 and 0.03 g kg^{-1} DM of sarsaponin	50:50	No effect	1.4 and −2.2%	Digestibility, TVFA, A/P unaffected,	Sliwinski et al. (2002)
Tea saponins	Lamb (60 days)	3 g day^{-1} or 4.1 g kg^{-1} diet	60:40	27.2%	28.3%[c]	TVFA increased; A/P unaffected; methanogen numbers decreased	Mao et al. (2010)
Tea saponins	Sheep (21 days)	5 g day^{-1} or 5 g kg^{-1} diet	60:40	8.71%	–	TVFA and A/P unaffected	Yuan et al. (2007)
Yucca schidigera extract (8 to 10% saponins)	Sheep (18 days)	13.8 g kg^{-1} diet or 16.4 g day^{-1}	60:40	11.7% (no effect)	15.6%	TVFA decreased, digestibility and A/P unaffected	Pen et al. (2007)
Y. schidigera plant (6% saponins)	Dairy cows (28 days)	10 g kg^{-1} of DM	51:49	No effect	2.5%	Digestibility, TVFA and A/P unaffected	Holtshausen et al. (2009)

Table 4 (continued)

Phytochemicals	Animals (duration)	Dosage	Diet (R/C)	Methane inhibition[a]	Methane inhibition[b]	Comments	References
Tannins							
Acacia mearnsii extract (CT 72.5%)	Sheep (21 days)	41 g kg^{-1} diet	50:50	9.9%	7.0%	Digestibility and TVFA unaffected, A/P decreased	Carulla et al. (2005)
A. mearsii tannins	Cattle (14 days)	8.6 and 14.6 g kg^{-1} of DM intake	Grazed on pasture with 4.5 kg grain/day	17.0 and 30%	13.9 and 22.4%[d]	Digestibility decreased	Grainger et al. (2009)
A. mearsii tannins	Cattle (35 days)	8.6 and 14.6 g kg^{-1} of DM intake	Grazed on pasture with 4.5 kg grain day^{-1}	11.5 and 28%	8.1 and 20.2%[d]	Digestibility decreased	Grainger et al. (2009)
Castanea sativa wood extract (contains HT 20%)	Sheep (21 days)	5 and 10.1 g kg^{-1} DM equivalent to 1 and 2 g kg^{-1} pure tannins	50:50	−21.5 (at 10.1 g kg^{-1}) to 32.6% (at 5 g kg^{-1})	−17.9 and −21.7%	Digestibility, TVFA, A/P unaffected	Sliwinski et al. (2002)
Terminalia chebula seed pulp	Sheep (35 days)	10 g kg^{-1} DM intake	50:50	No effect	24.0%	Digestibility increased	Patra et al. (2010b)
Essential oils							
Cinnamaldehyde	Sheep (91 days)	0.02 g kg^{-1} diet	Barley-based diet	–	–	No effect on methanogenic counts, increased diversity of methanogens	Ohene-Adjei et al. (2008)
Juniper berry oil [*Juniperus communis*]	Sheep (91 days)	0.02 g kg^{-1} diet	Barley-based diet	–	–	No effect on methanogen numbers, increased the diversity of methanogens	Ohene-Adjei et al. (2008)

R/C roughage to concentrate ratio, *TVFA* total volatile fatty acids concentration, *DM* dry matter, *A/P* acetate to propionate ratio, *CT* condensed tannins, *HT* hydrolysable tannins

[a] Inhibition of methane production compared with control (without phytochemicals) on volume basis

[b] Inhibition of methane production compared with control (without phytochemicals) relative to dry matter/organic matter digested unless other wise marked

[c] Relative to per kilogram of body weight gain

[d] Relative to per kilogram of milk yield

et al. (2006) reported that the depression in CH_4 production was 96%, 39.4%, and 20% with ethanol, water, and methanol extracts of seed pulps of *Sapindus murkossi*, respectively, compared with controls. However, saponins extracted from pods of *Acacia concinna* extracts did not affect CH_4 production in 1:1 concentrate to roughage-based diet despite a depression in protozoal numbers (Patra et al. 2006a). It has been observed that effect of *S. saponaria* on CH_4 was more pronounced in defaunated (29%) than faunated (14%) rumen fluid indicating that reduced CH_4 production was not entirely due to associated depression in protozoal numbers (Hess et al. 2003). The inhibitory activities of some saponins on methanogenesis are dependent on the composition of diets and levels of saponins in the diets. For example, saponins of *Sapindus rarak* fruits reduced methanogen RNA concentration at the highest saponins concentration (4 mg ml^{-1}), while lower levels had no effect on methanogens numbers (Wina et al. 2005). Goel et al. (2008) noted that CH_4 inhibition effect of saponins from *Sesbania sesban* and fenugreek was pronounced in concentrate-based diets compared with roughage-based diets. Total archaeal population was reduced by saponins extracted from *S. sesban* leaves (78%), fenugreek seeds (22%), and *Knautia* leaves (21%). Despite inhibition of archaea, CH_4 production was not affected in their study (Goel et al. 2008), which might be due to changes in the rate of methanogenesis as a result of changing fermentation pattern and microbial diversity.

One of the problems of using saponins or saponin-containing plants is that anti-protozoal activity was found to be transient (Patra and Saxena 2009). Protozoa did not become resistant to these anti-protozoal compounds (Newbold et al. 1997). Therefore, it is possible that bacterial populations of the rumen degraded the saponins or saponin-containing plants (Newbold et al. 1997; Patra and Saxena 2009). These studies are providing evidence that rumen-mixed microbial populations are able to adapt to saponins over time, which present a challenge for practical application of this feed additive technology. Nonetheless, in addition to suppressing methane outputs, the use of saponins may also confer nutritional benefits as they might increase microbial protein synthesis due to inhibition of protozoa, and the fiber-degrading bacteria and fungi in the rumen might increase, which is beneficial for utilization of feeds in low-quality-based diets (Patra and Saxena 2009).

Tannins Different sources of tannin extracts have been shown to decrease CH_4 production both in vitro and in vivo condition depending upon doses. Addition of *Acacia mearnsii* tannin extracts suppressed CH_4 production in sheep by 10% (Carulla et al. 2005) and in cattle up to 30% (Grainger et al. 2009) decreased methanogenesis. Methane production was also inhibited by inclusion of methanol extract of pericarp of *Terminalia chebula* (a tropical fruit) in vitro up to 90% (Patra et al. 2006a) and in sheep fed 10 g kg^{-1} of DM intake (Patra et al. 2010b), which could be due to the presence of tannins especially hydrolysable tannins in these fruits. Min et al. (2005) found that quebracho tannin (75% CT) included at concentrations of 1 to 2 g L^{-1} decreased CH_4 production by 12.3% to 32.6% in an in vitro condition. Similarly, feeding of quebracho tannins at 10–20 g kg^{-1} DM intake to cattle grazing wheat grass in reproductive stage with rumen liquor collected from them for testing CH_4 production in vitro caused a decrease in CH_4 production by 25% to 51% (Min et al. 2006). But cattle grazing wheat grass in vegetative stage did not exhibit anti-methanogenic effect in this study (Min et al. 2006). More recently, Bhatta et al. (2009) reported that quebracho tannins inhibited the CH_4 production linearly (13% to 45%) with increasing doses (5% to 25% of substrates). However, Beauchemin et al. (2007) did not find any effect on methanogenesis when a quebracho tannin extract (10–20 g kg^{-1} DM intake) was fed to beef cattle, which may be due to low dosages of tannins. It has been suggested that the action of CT on methanogenesis may be attributed to the direct inhibitory effects on methanogens depending upon the chemical structure of CT and also indirectly by decreasing fiber degradation (Patra and Saxena 2010).

Essential oils and organosulfur compounds A number of reports are available showing

abatement of CH_4 production by essential oils (EO) and organosulfur compounds. Evans and Martin (2000) observed that thymol (400 mg L^{-1}), a main component of EO derived from *Thymus* and *Origanum* plants, was a strong inhibitor of CH_4 in vitro, but acetate and propionate concentrations also decreased. Methanol and ethanol extracts of *Foeniculum vulgare* and *Syzygium aromaticum* inhibited CH_4 production in vitro (Patra et al. 2006b, 2010a), which was also accompanied by reduction of degradability of feeds by *S. aromaticum*, whereas the extracts of *A. sativum* and *F. vulgare* had no effects on degradability of feeds (Patra et al. 2010a). With organosulfur compounds, i.e., garlic oil and four of its main components (diallyl sulfide, diallyl disulfide, allyl mercaptan, and allicin), Busquet et al. (2005) observed that garlic oil and diallyl disulfide (300 mg L^{-1} of ruminal fluid) reduced CH_4 production by 74% and 69%, respectively, without altering digestibility of nutrients in batch cultures. Busquet et al. (2005) suggested that garlic oil and diallyl disulfide might have inhibited CH_4 production due to the direct inhibition of rumen methanogenic archaea. In an experiment with sheep fed on wheat straw and concentrate (1:1), inclusion of *Allium sativum* at 10 g kg^{-1} of DM intake also reduced CH_4 production per unit of OM digested and increased digestibility of fiber (Patra et al. 2010b).

A limited number of studies are available showing direct effect of EO on rumen archaea. In a culture-based study, EO did not inhibit *Methanobrevibacter smithii* up to a concentration of 0.16 ml L^{-1} although inhibition occurred at 1.0 ml L^{-1} (McIntosh et al. 2003). Ohene-Adjei et al. (2008) also reported that cinnamaldehyde, garlic, and juniper oil supplementation in barley-based diet did not affect total number of methanogenic archea quantified by arecheal 16S rRNA. Interestingly, the phylogenetic analysis indicated that cinnamaldehyde, garlic, and juniper oil supplementation reduced the proportion of clones associated within the *M. ruminantium*-related cluster, which was more pronounced for juniper berry oil supplementation. Conversely, clones affiliated to *Methanosphaera stadtmanae* and *M. smithii* and some uncultured groups increased in the supplemented treatments. This suggested that EO increased the phylogenetic distribution of methanogenic archaea, which may have resulted from changes in associated protozoal species (Ohene-Adjei et al. 2008). Agarwal et al. (2009) reported that inclusion of 0.33 ml/L of peppermint oil increased methanogen numbers by two-fold although there was a decrease in CH_4 production by 20% without affecting volatile fatty acid production. In this study, the higher levels (1 and 2 ml L^{-1}) of peppermint oil decreased total methanogen population and CH_4 production. It appears that a decrease in methanogenesis at low doses might be associated with the changes in the rate of methanogenesis by archaea due to the alteration of archaeal community or in the activity of CH_4-producing genes (Ohene-Adjei et al. 2008). Overall, although phytochemicals look promising in suppressing CH_4 emissions in ruminants, results are not consistent in different studies because of great variations in chemical composition of phytochemicals, doses, and feed composition (Patra and Saxena 2010). A great deal of research would be needed based on structure–activity relationship for practical application of phytochemicals.

Defaunation

Removal of protozoa (defaunation) from the rumen is often associated with an increased microbial protein supply and improvement of animal productivity (Patra and Saxena 2009). Besides, many methanogens remain attached on the exterior surface of rumen ciliate protozoa and as endosymbionts within the ciliates, which are responsible for up to 37% of rumen methanogenesis (Finlay et al. 1994). Hence, defaunation has been suggested as a way to reduce CH_4 production with little or minimal effect on rumen digestion. Morgavi et al. (2008) showed that CH_4 emission decreased by 20% for a period of 2 years in defaunated sheep. However, partial defaunation is not always found to be effective in decreasing CH_4 production; the reason of which is unclear (Patra et al. 2006a; Hegarty et al. 2008). A variety of techniques for defaunation have been tested experimentally, but none is used routinely

because of toxicity problems to the rest of the rumen microbial population and the host animals (Moss et al. 2000). In recent years, there has been an increased interest for use of plant secondary metabolites as potential defaunating agents. In particular, saponin-containing plants look promising as a possible mean of suppressing or eliminating protozoa in the rumen without inhibiting bacterial activity (Agarwal et al. 2006; Patra and Saxena 2009). Recently, it has been reported that vaccination of sheep with entodinial or mixed protozoal antigens reduced protozoal numbers, and IgG antibodies generated against rumen protozoa remained active and continued to bind target cells for up to 8 h (Williams et al. 2008). Defaunation technology needs further assessment for practical delivery at farmers' fields.

Immunization against methanogens

In order to inhibit methanogens without affecting useful ruminal microbes, it is essential to have methanogen-specific targets for inhibitors. Australian researchers demonstrated for the first time that the vaccination against methanogens may be another plausible method for mitigating CH_4 emission (Wright et al. 2004). Immunization of sheep with a mixed whole-cell preparation from three methanogens reduced CH_4 production by 7.7% (grams per kilogram of DM intake). However, immunization with a mixed whole-cell preparation from seven methanogens did not affect CH_4 production in sheep (Wright et al. 2004). Canadian researchers prepared IgY antibodies in chicken eggs against methanogens generated by inoculating hens with whole-cell preparations of three species of methanogens (Cook et al. 2008). When egg powder containing anti-methanogen antibodies was added in in vitro batch cultures, CH_4 production reduced at 12 h of incubation but not at 24 h of incubation. This result suggests that antibodies may only have a transient influence on methanogens, possibly due to degradation of antibodies or diversification of methanogen population (McAllister and Newbold 2008). Because the diversity of methanogens may be influenced by diets and geographic location, it is a challenge to prepare broad spectrum vaccines that will be effective across different production systems of geographically diverse regions.

Archaeal-specific genes and cell surface proteins in *M. ruminantium* and other methanogens could be an area of research to discover potential targets for CH_4 mitigation and methanogen vaccine development (Atwood and McSweeney 2008). More recently, Leahy et al. (2010) identified several gene targets to inhibit CH_4 in *M. ruminantium* via chemogenomic and vaccine approaches and showed that vaccinations of sheep with synthetic peptides against some gene targets raised antibody titers in serum. Vaccination of sheep with subcellular fractions such as cytoplasmic and cell wall preparations, and cell wall-derived proteins or whole cells of *M. ruminantium* augmented antibody in the sera against methanogens, and antibodies inhibited the growth of *M. ruminantium* and CH_4 production in vitro (Wedlock et al. 2010). Development of a recombinant vaccine against methanogens' cell surface proteins that are conserved across a broad range of methanogen species may be successful as a CH_4 mitigation technique in future.

Use of bacteriocins

Bacteriocins are bacterial proteins or peptides produced by bacteria and play a role in competition among microbial species for niches within the ruminal ecosystem. Bovicin HC5, a bacteriocin produced by *Streptococcus* species from the rumen, was reported to suppress CH_4 production in vitro by 50% (Lee et al. 2002). Nisin, a bacteriocin from *Lactobacillus lactis* subsp. *lactis*, has also been shown to decrease CH_4 production in vitro. Combinations of nisin and nitrate, an alternative electron acceptor, have been reported to lessen CH_4 emissions in sheep (Sar et al. 2005). The use of bacteriocins may be prospective for inhibiting methanogen populations in the rumen.

Bacteriophage therapy

Bacteriophages are microbial viruses that infect both bacteria and methanogens and lyse their host cells during the lytic phase of their development. Rumen bacteriophages are present in high

numbers ($>10^9$ ml^{-1}) in rumen fluid. Possibly until now, no phages specific to rumen methanogens have been isolated from rumen fluid (McAllister and Newbold 2008). However, phages with activity against other rumen bacteria and non-rumen methanogens have been reported. Identification of rumen phages against methanogens that possess activity specifically against methanogens might be an area of exploration.

Alternate hydrogen sinks

Propionate enhancers

A decrease in CH_4 production up to 20–50% by suppression of methanogens could be achievable without reducing feed intake and body weight gain and could increase energetic efficiency to 2–5% of digestion (Atwood and McSweeney 2008). However, utilization of H_2 through alternative avenues should be considered to ameliorate the depression of fiber digestion in the rumen when methanogens are inhibited. Use of propionate enhancers and other electron acceptors and stimulation of reductive acetogenesis appear to be promising for disposal of H_2 Addition of organic acids that are intermediates of propionate formation such as malate and fumarate increases propionate production with a stoichiometric decrease in H_2 availability for CH_4 production. Kolver et al. (2004) noted a 38% lower in CH_4 production when fumarate was added at a dose level of 3.5 g L^{-1} in continuous fermenters with forages as a substrate. Acrylate, an alternative precursor of propionate, also depresses CH_4 production in rumen, but to a lesser extent than an equimolar addition of fumarate. Similarly, increasing concentrations of malate (0%, 3.75%, and 7.5% of DM intake) in the diet of beef cattle lowered daily CH_4 emissions linearly with a decrease of 16% at the highest dosage, which corresponded to a 9% reduction per unit of DM intake (Foley et al. 2009). However, the results are not consistent depending upon the dose levels and diets (Foley et al. 2009). For example, CH_4 emissions were not affected by addition of fumarate (10 g kg^{-1}) in the diet of beef cattle (Beauchemin and McGinn 2006), while CH_4 outputs from sheep were decreased by feeding of higher level of fumarate (10% of diet) to the extent of 40% to 75% per kg of DM intake, and there was an improvement of animal performance (Wallace et al. 2006). The inconsistency of these acids on CH_4 production might be due to the conversion of these acids to acetate instead of propionate that stoichiometrically may increase CH_4 production in the rumen (Ungerfeld et al. 2007). In addition, methanogenic microorganisms can predominate over fumarate reducing bacteria at low hydrogen concentrations normally present in the rumen because the affinity of fumarate-utilizing bacteria to H_2 may be lower than the affinity of methanogens (Asanuma et al. 1999). Therefore, it is necessary to identify physiological and biochemical conditions, which could favor propionate rather than acetate production from these organic acids (Atwood and McSweeney 2008).

Besides inconsistent results, propionate enhancers generally are required at high doses to lessen CH_4, which makes this an expensive technology. These organic acids are found in leaves of forages, and malate can account for about 6–7% of DM of lucerne forage in immature stage, which declines rapidly with the maturity of plants resulting malate concentrations of 3% to 4.5% at day 42 (Callaway et al. 1997; Martin 1998). It has been suggested that selection of forages for high malate content and the plant breeding programs to enhance the concentrations of this organic acid in forages may be economically reasonable for inclusion of malate in the diets (Martin 1998) and, hence, for the CH_4 mitigation technology.

Alternative electron acceptors

The methanogenesis could also be suppressed by increasing the utilization of H_2 by organisms other than methanogens. Some rumen microorganisms capable of reduction of nitrate to nitrite and then nitrite to ammonia use hydrogen or formate or both as the common electron donors; thus, methanogenesis may be lowered by the addition of electron acceptors such as nitrate and sulfate (Sar et al. 2004a, b).

$$NO_3^- + 2H^+ \rightarrow H_2O + NO_2^-$$

$$NO_2^- + 6H^+H_2O + NH_3$$

$$3HCO_2^- + NO_2^- + 5H^+CO_2 + NH_4^- + 2H_2O$$

The end product of sulfate metabolism, i.e., hydrogen sulfide could be toxic, but nitrate could be preferably utilized as an electron acceptor since the end product of nitrate metabolism by rumen microbes is ammonia. It has been generally suggested that CH_4 production could be diminished by 10% for each 1% inclusion of potassium nitrate in a diet (Leng 2008). Another advantage of using nitrate is that it could be used as a nitrogen supplement to low-quality crop residue-based diets. In a study of Sar et al. (2004b), feeding of sodium nitrate (1.3 g kg^{-1} $BW^{0.75}$) for 7 days to sheep suppressed CH_4 production by 50% and the ammonia concentration in the rumen increased. However, under some nutritional conditions/feed management, nitrate becomes toxic because of the accumulation of nitrite in the rumen. It has been suggested that the application of nitrate could decrease enteric CH_4 production by 50%, and toxicity problems could be reduced by changing the production management to low protein diets and a gradual introduction of nitrate to animals (Leng 2008).

Stimulation of acetogens

An alternative strategy to reduce ruminal methanogenesis could be to redirect H_2 from methanogens to acetogens by reductive acetogenesis pathway.

Lopez et al. (1999) found that acetogens depressed CH_4 production when added to rumen fluid in vitro. Research indicated that some selected acetogens can lower H_2 concentration when methanogenesis is inhibited in vitro (LeVan et al. 1998). Therefore, acetogens might also be promising alternative sink for H_2 in the rumen once CH_4 mitigation strategies are applied. It is suggested that a decrease in H_2 concentration in the rumen using the acetogens as a daily fed feed additive, even a stable population of acetogens could not be established in the rumen (Lopez et al. 1999).

Inclusion of probiotic cultures

Probiotics are used in the diets of ruminant to improve health status, rumen fermentation, and animal performance, which could also cut down CH_4 emissions as discussed earlier. While there are many studies on rumen fermentation and animal performance, limited information is available on the effect of probiotic cultures such as *Saccharomyces cerevisiae* and *Aspergillus oryzae* on CH_4 production and most of all are in vitro. Addition of *S. cerevisiae* to an in vitro system suppressed CH_4 formation by 10% initially, though this was not sustained (Mutsvangwa et al. 1992). Lynch and Martin (2002) reported a 20% decrease in CH_4 after 48 h of incubation of mixed rumen microorganisms in the presence of alfalfa and a live yeast product. *A. oryzae* has been found to lower CH_4 production to the extent of 50% (Frumholtz et al. 1989), which was directly related to a reduction in the protozoal population (45%). In some experiments, *A. oryzae* and *S. cerevisiae* increased CH_4 production (Martin et al. 1989; Martin and Nisbet 1990), while Mathieu et al. (1996) reported that *S. cerevisiae* addition did not affect CH_4 release in vivo. Mwenya et al. (2004) reported that a yeast culture containing *Trichosporon sericeum* (4 g day^{-1}) depressed CH_4 production by 10% in sheep fed on a roughage-based diet. It is suggested that yeast culture probably stimulates the acetogens to compete with methanogens or to co-metabolize H_2 thus decreasing CH_4 formation (Chaucheyras et al. 1995; Mwenya et al. 2004). These conflicting results on CH_4 production might be due to strain differences between yeast cultures and type of diets (Newbold and Rode 2006). Thus, selection of probiotic strains for the CH_4-suppressing effect could be attempted. This suggests that more research is required before it can be recommended that yeast cultures can decrease CH_4 production in ruminants.

Rumen methane oxidation

Microbial oxidation of CH_4 to CO_2 and H_2 by CH_4 oxidizing bacteria (methanotrophs) in the rumen has been proposed to reduce enteric CH_4 production. Methanotrophs (Proteobacteria) have been isolated from a wide range of environments, including the rumen, but there has been little investigation on physiology and molecular evidence of their role in methanotrophy in the rumen (Mitsumori et al. 2002). Some in vitro studies with rumen fluid suggest that oxidation of CH_4 to CO_2 is of little quantitative significance (0.2–0.5% of

total CH_4 produced) in the rumen (Kajikawa et al. 2003). The importance of these methanotrophs in the rumen warrants further investigation in terms of their novelty and practical implication in reducing CH_4 emissions from ruminants (Atwood and McSweeney 2008).

Conclusions

A large number of the potential options discussed above have only been tested experimentally, and thus need more research to confirm their prospective contributions to curb CH_4 emissions in field conditions. Farmers are unlikely to adopt abatement technologies unless there are positive impacts on cost-effective animal production. The abatement strategies that improve feed efficiency or productivity such as using high-quality forages, supplementation with concentrates and green fodders, use of monensin and fats, incorporation of CT-containing forages, and maintenance of high-producing animals in the herds are more likely to be encouraging to the farmers with the present technologies. In this way, CH_4 emissions per unit of product can be reduced, and total animal production can be increased. Accounting the cost of technology and the price of the product gain, Sirohi et al. (2007) analyzed that supplementation of diets with monensin (rumensin), concentrates, and urea molasses mineral block could be cost-effective for high-yielding animals in Indian situation.

A number of the technologies such as use of plant secondary metabolites, probiotics, and organic acids, stimulation of acetogens, and immunization against methanogens have emerged to lower CH_4 production. However, most of these have not been tried in long-term experiments in different nutritional feeding management systems and thus require extensive research. The CH_4 oxidation by methylotrophs, use of bacteriocins and bacteriophases, and development of recombinant vaccines targeting archaea-specific genes and cell surface proteins might be an area worthy of investigation for CH_4 mitigation. Evidently, comprehensive research is needed to develop CH_4 mitigation technologies that will provide consistent results. Simultaneously, a broad understanding of both the rumen microbial ecology and methanogen biochemistry are required for successful achievement of CH_4 mitigation.

There are concerns that inhibiting the CH_4 production in the rumen may increase the CH_4 emission from manure. For example, reductions in enteric CH_4 emission in cow fed lauric acid was largely compensated by increases in CH_4 emission from manure (Kulling et al. 2002). Similarly, decreases in enteric CH_4 per kilogram of dry matter intake (−18%) were diminished to −12% of total CH_4 via the opposite trend in slurry methanogenesis (Hindrichsen et al. 2006). In contrast, CH_4 inhibition in the rumen may also decrease CH_4 production from manure during composting. Supplementation with *A. mearsii* CT in the diet of cattle decreased CH_4 production by 23% during manure composting (Hao et al. 2010). This could be a concern for generation of CH_4 as biogas using manure from these animals in biodigesters. However, it is argued that lowering enteric CH_4 emission could be considered profitable since this CH_4 is inevitably lost whereas manure storage technology or anaerobic digester processes could offer opportunities to avoid high CH_4 losses or produce CH_4 as biogas (Kulling et al. 2002). Nevertheless, an integrated and holistic approach should be taken into deliberation for CH_4 mitigation depending upon the manure management systems.

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References

Agarwal, N., Kamra, D. N., Chaudhary, L. C., & Patra, A. K. (2006). Effect of *Sapindus mukorossi* extracts on in vitro methanogenesis and fermentation characteristics in buffalo rumen liquor. *Journal of Applied Animal Research, 30*, 1–4.

Agarwal, N., Shekhar, C., Kumar, R., Chaudhary, L. C., & Kamra, D. N. (2009). Effect of peppermint (*Mentha piperita*) oil on in vitro methanogenesis and fermentation of feed with buffalo rumen liquor. *Animal Feed Science and Technology, 148*, 321–327.

Alford, A. R., Hegarty, R. S., Parnell, P. F., Cacho, O. J., Herd, R. M., & Griffith, G. R. (2006). The impact of breeding to reduce residual feed intake on enteric methane emission from the Australian beef industry.

Australian Journal of Experimental Agriculture, 46, 813–820.

Anderson, R. C., Callaway, T. R., Van Kessel, J. S., Jung, Y. S., Edrington, T. S., & Nisbet, D. J. (2003). Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. *Bioresource Technology, 90*, 59–63.

Anderson, R. C., Carstens, G. E., Miller, R. K., Callaway, T. R., Schultz, C. L., Edrington, T. S., et al. (2006). Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen. *Bioresource Technology, 97*, 2421–2426.

Anderson, R. C., Krueger, N. A., Stanton, T. B., Callaway, T. R., Edrington, T. S., Harvey, R. B., et al. (2008). Effects of select nitrocompounds on in vitro ruminal fermention during conditions of limiting or excess added reductant. *Bioresource Technology, 99*, 8655–8661.

Animut, G., Goetsch, A. L., Puchala, P., Patra, A. K., Sahlu, T., Varel, V. H. et al. (2008a). Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. *Animal Feed Science and Technology, 144*, 212–227.

Animut, G., Goetsch, A. L., Puchala, P., Patra, A. K., Sahlu, T., Varel, V. H. et al. (2008b). Methane emission by goats consuming different sources of condensed tannins. *Animal Feed Science and Technology, 144*, 228–241.

Asanuma, N., Iwamoto, M., & Hino, T. (1999). Effect of the addition of fumarate on methane production by ruminal microorganisms in vitro. *Journal of Dairy Science, 82*, 780–787.

Atwood, G., & McSweeney, C. S. (2008). Methanogen genomics to discover targets for methane mitigation technologies and options for alternative H_2 utilization in the rumen. *Australian Journal of Experimental Agriculture, 48*, 28–37.

Beauchemin, K. A., & McGinn, S. M. (2006). Methane emissions from beef cattle: Effects of fumaric acid, essential oil and canola oil. *Journal of Animal Science, 84*, 1489–1496.

Beauchemin, K. A., McGinn, S. M., & Petit, H. V. (2007). Methane abatement strategies for cattle: Lipid supplementation of diets. *Canadian Journal of Animal Science, 87*, 431–440.

Beauchemin, K. A., Kreuzer, M., O'Mara, F., & McAllister, T. A. (2008). Nutritional management for enteric methane abatement: A review. *Australian Journal of Experimental Agriculture, 48*, 21–27.

Beauchemin, K. A., McGinn, S. M., Benchaar, C., & Holtshausen, L. (2009). Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. *Journal of Dairy Science, 92*, 2118–2127.

Benchaar, C., Pomar, C., & Chiquette, J. (2001). Evaluation of diet strategies to reduce methane production in ruminants: A modelling approach. *Canadian Journal of Animal Science, 81*, 563–574.

Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., et al. (2009). Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science, 92*, 5512–5522.

Bowman, R. L., Croucher, J. C., & Picard, M. T. (1992). *Assessment of the Pre-feasibility of Strategic Supplementation as an Opportunity for Reducing Methane Emissions in Gujarat, India*. Washington, D.C.: Global Change Division, U.S. Environmental Protection Agency.

Busquet, M., Calsamiglia, S., Ferret, A., Carro, M. D., & Kamel, C. (2005). Effect of garlic oil and four of its compounds on rumen microbial fermentation. *Journal of Dairy Science, 88*, 4393–4404.

Callaway, T. R., Martin, S. A., Wampler, J. L., Hill, N. S., & Hill, G. M. (1997). Malate content of forage varieties commonly fed to cattle. *Journal of Dairy Science, 80*, 1651–1655.

Carulla, J. E., Kreuzer, M., Machmuller, A., & Hess, H. D. (2005). Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agricultural Research, 56*, 961–970.

Chaucheyras, F. G., Fonty, G., Bertin, G., & Gouet, P. (1995). In vitro H_2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archaea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology, 61*, 3466–3467.

Cook, S. R., Maiti, P. K., Chaves, A. V., Benchaar, C., Beauchemin, K. A., & McAllister, T. A. (2008). Avian (IgY) anti-methanogen antibodies for reducing ruminal methane production: In vitro assessment for their effects. *Australian Journal of Experimental Agriculture, 48*, 260–264.

Dong, Y., Bae, H. D., MaAllister, T. A., Mathison, G. W., & Cheng, K. J. (1999). Effects of exogenous fibrolytic enzymes, 2-bromoethanesulfonate, and monensin on fermentation in rumen simulation (RUSITEC) system. *Canadian Journal of Animal Science, 79*, 491–498.

Eckard, R. J., Grainger, C., & de Klein, C. A. M. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: A review. *Livestock Science, 130*, 47–56.

Evans, J. D., & Martin, S. A. (2000). Effects of thymol on ruminal micro-organisms. *Current Microbiology, 41*, 336–340.

Finlay, B. J., Esteban, G., Clarke, K. J., Williams, A. G., Embley, T. M., & Hirt, R. R. (1994). Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letters, 117*, 157–162.

Foley, P. A., Kenny, D. A., Callan, J. J., Boland, T. M., & O'Mara, F. P. (2009). Effect of DL-malic acid supplementation on feed intake, methane emission, and rumen fermentation in beef cattle. *Journal of Animal Science, 87*, 1048–1057.

Frumholtz P. P., Newbold C. J., & Wallace R. J. (1989). Influence of *Aspergillus oryzae* fermentation extract on the fermentation of a basal ration in the rumen

simulation technique (Rusitec). *Journal of Agricultural Science (Cambridge), 113*, 169–172.

Garcia-Lopez, P. M., Kung, L. Jr., & Odom, J. M. I. (1996). In vitro inhibition of microbial methane production by 9,10- anthraquinone. *Journal of Animal Science, 74*, 2276–2284.

Goel, G., Makkar, H. P. S., & Becker, K. (2008). Effect of *Sesbania sesban* and *Carduus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrient from roughage and concentrate based feeds to methane. *Animal Feed Science and Technology, 147*, 72–89.

Grainger, C., Clarke, T., Beauchemin, K. A., McGinn, S. M., & Eckard, R. J. (2008). Supplementation with whole cottonseed reduces methane emissions and increases milk production of dairy cows offered a forage and cereal grain diet. *Australian Journal of Experimental Agriculture, 48*, 73–76.

Grainger, C., Clarke, T., Auldist, M. J., Beauchemin, K. A., McGinn, S. M., Waghorn, G. C. et al. (2009). Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science, 89*, 241–251.

Guan, H., Wittenberg, K. M., Ominski, K. H., & Krause, D. O. (2006). Efficacy of ionophores in cattle diets for mitigation of enteric methane. *Journal of Animal Science, 84*, 1896–1906.

Gutierrez-Bañuelos, H., Anderson, R. C., Carstens, G. E., Slay, L. J., Ramlachan, N., Horrocks, S. M. et al. (2007). Zoonotic bacterial populations, gut fermentation characteristics and methane production in feedlot steers during oral nitroethane treatment and after the feeding of an experimental chlorate product. *Anaerobe, 13*, 21–31.

Hao, X., Benke, M. B., Li, C., Larney, F. J., Beauchemin, K. A., & McAllister, T. A. (2010). Greenhouse gas emissions during composting of manure from cattle diets including corn dried distillers grains with solubles and condensed tannins. In *Proceedings of abstracts, Greenhouse Gases and Animal Conference*, October 3–8, 2010, Canada, p.30.

Haque, N., Saraswat, M. L., & Sahoo, A. (2001). Methane production and energy balance in crossbred male calves fed on rations containing different ratios of green sorghum and wheat straw. *Indian Journal of Animal Sciences, 71*, 797–799.

Hegarty, R. S., Goopy, J. P., Herd, R. M., & McCorkell, B. (2007). Cattle selected for lower residual feed intake have reduced daily methane production. *Journal of Animal Science, 85*, 1479–1486.

Hegarty, R. S., Bird, S. H., Vanselow, B. A., & Woodgate, R. (2008). Effects of the absence of protozoa from birth or from weaning on the growth and methane production of lambs. *British Journal of Nutrition, 100*, 1220–1227.

Hess, H. D., Monsalve, L. M., Lascano, C. E., Carulla, J. E., Diaz, T. E., & Kreuzer, M. (2003). Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: Effects on in vitro ruminal nitrogen turnover and methanogenesis. *Australian Journal of Agricultural Research, 54*, 703–713.

Hess, H. D., Beuret, R. A., Lotscher, M., Hindrichsen, I. K., Machmüller, A., Carulla, J.E. et al. (2004). Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with *Sapindus saponaria* fruits and *Cratylia argentea* foliage. *Animal Science, 79*, 177–189.

Hindrichsen, I. K., Wettstein, H. -R., Machmuller, A., & Kreuzer, M. (2006). Methane emission, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different milk production scenarios with and without concentrate supplementation. *Agriculture, Ecosystems and Environment, 113*, 150–161.

Holter, J. E., Hayes, H. H., & Urban, W. E. Jr. (1992). Energy balance and lactation response in Holstein cows supplemented with cottonseed with or without calcium soap. *Journal of Dairy Science, 75*, 1480–3494.

Holtshausen, L., Chaves, A. V., Beauchemin, K. A., McGinn, S. M., McAllister, T. A., Odongo, N. E., Cheeke, P. R., &Benchaar, C. (2009). Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. *Journal Dairy Science, 92*, 2809–2821.

Hook, S. E., Northwood, K. S., Wright, A. -D. G., & McBride, B. W. (2009). Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of the lactating dairy cow. *Applied and Environmental Microbiology, 75*, 374–380.

IPCC (2007). Summary for Policymakers. In Solomon, S. D., Qin, M., Manning, Z., Chen, M., Marquis, K. B, Averyt, M. T., & Miller, H. L. (Eds.), *Climate change 2007: The physical science basis. Contribution of working group i to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge.

Janssen, P. J., & Kirs, M. (2008). Structure of the archaeal community of the rumen. *Applied and Environmental Microbiology, 74*, 3619–3625.

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*, 2483–2492.

Jordan, E., Lovett, D. K., Hawkins, M., Callan, J. J., & O'Mara, F. P. (2006a). The effect of varying levels of coconut oil on intake, digestibility and methane output from continental cross beef heifers. *Animal Science, 82*, 859–865.

Jordan, E., Lovett, D. K., Monahan, F. J., Callan, J., Flynn, B., & O'Mara, F. P. (2006b). Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *Journal of Animal Science, 84*, 162–170.

Jordan, E., Kenny, D., Hawkins, M., Malone, R., Lovett, D. K., & O'Mara, F. P. (2006c). Effect of refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. *Journal of Animal Science, 84*, 2418–2425.

Joyner, A. E. Jr., Brown, L. J., Fogg, T. J., & Rossi, R. T. (1979). Effect of monensin on growth, feed efficiency

and energy metabolism of lambs. *Journal of Animal Science, 48*, 1065–1069.

Kajikawa, H., Valdes, C., Hillman, K., Wallace, R. J., & Newbold, C. J. (2003). Methane oxidation and its coupled electron-sink reactions in ruminal fluid. *Letters in Applied Microbiology, 36*, 354–357.

Kamra, D. N., Patra, A. K., Chatterjee, P. N., Kumar, R., Agarwal, N., & Chaudhary, L. C. (2008). Effect of plant extract on methanogenesis and microbial profile of the rumen of buffalo: A brief overview. *Australian Journal of Experimental Agriculture, 48*, 175–178.

Klita, P. T., Mathison, G. W., Fenton, T. W., & Hardin, R. T. (1996). Effects of alfalfa root saponins on digestive function in sheep. *Journal of Animal Science, 74*, 1144–1156.

Kolver, E. S., Aspin, P. W., Jarvis, G. N., Elborough, K. M., & Roche, J. R. (2004). Fumarate reduces methane production from pasture fermented in continuous culture. *Proceedings of the New Zealand Society of Animal Production, 64*, 155–159.

Kulling, D. R., Dohme, F., Menzi, H., Sutter, F., Lischer, P., & Kreuzer, M. (2002). Methane emissions of differently fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. *Environmental Monitoring and Assessment, 79*, 129–150.

Kung, L. Jr., Smith, K. A., Smagala, A. M., Endres, K. M., Bessett, C. A., Ranjit, N. K. et al. (2003). Effects of 9,10-anthraquinone on ruminal fermentation, total-tract digestion, and blood metabolite concentrations in sheep. *Journal of Animal Science, 81*, 323–328.

Leahy, S. C., Kelly, W. J., Altermann, E., Ronimus, R. S., Yeoman, C. J., Pacheco, D. M. et al. (2010). The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. *PLoS One, 5*, e8926. doi:10.1371/journal.pone.0008926

Lee, S. S., Hsu, J. T., Mantovani, H. C., & Russell, J. B. (2002). The effect of bovicin HC5, a bacteriocin from *Streptococcus bovis* BC5, on ruminal methane production in vitro. *FEMS Microbiology Letters, 217*, 51–55.

Leng, R. A. (2008). The potential of feeding nitrate to reduce enteric methane production in ruminants. A Report to the Department of Climate Change, Commonwealth Government of Australia Canberra ACT Australia, pp. 90.

LeVan T. D., Robinson, J. A., Ralph, J., Greening, R. C., Smolenski, W. J., Leedle, J. A. Z. et al. (1998). Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and *Acetomaculum ruminis*. *Applied and Environmental Microbiology, 64*, 3429–2436.

Lila, Z. A., Mohammed, N., Tatsuoka (Ajisaka), N., Kanda, S., Kurokawa, Y., & Itabashi, H. (2004). Effect of cyclodextrin diallyl maleate on methane production, ruminal fermentation and microbes in vitro and in vivo. *Animal Science Journal, 75*, 15–22.

Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of New York Academy of Sciences, 1125*, 171–189.

Lopez, S. M., McIntosh, F. M., Wallace, R. J., & Newbold, C. J. (1999). Effect of adding acetogenic bacteria on methane production by mixed rumen micro-organisms. *Animal Feed Science and Technology, 78*, 1–9.

Lovett, D. K., Lovell, S., Stack, L., Callan, J., Finlay, M., Conolly, J. et al. (2003). Effect of forage/concentrate ratio and dietary coconut oil level on methane output and performance of finishing beef heifers. *Livestock Production Science, 84*, 135–146.

Lynch, H. A., & Martin, S. A. (2002). Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation. *Journal of Dairy Science, 85*, 2603–2608.

Machmüller, A., & Kreuzer, M. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science, 79*, 65–72.

Machmüller, A., Soliva, C. R., & Kreuzer, M. (2003a). Effect of coconut oil and defaunation treatment on methanogenesis in sheep. *Reproduction Nutrition and Development, 43*, 41–55.

Machmüller, A., Soliva, C. R., & Kreuzer, M. (2003b). Methane suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. *British Journal of Nutrition, 90*, 529–540.

Mao, H. -L., Wang, J. -K., Zhou, Y. -Y., & Liu, J. -X. (2010). Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. *Livestock Science, 129*, 56–62.

Martin, S. A. (1998). Manipulation of ruminal fermentation with organic acids: A review. *Journal of Animal Science, 76*, 3123–3132.

Martin, S. A., & Nisbet, D. J. (1990). Effects of Aspergillus oryzae fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal micro-organisms in vitro. *Journal of Animal Science, 68*, 2142–2149.

Martin S. A., Nisbet, D. J., & Dean, R. G. (1989). Influence of a commercial yeast supplement on the in vitro ruminal fermentation. *Nutrition Reproduction International, 40*, 395–403.

Martin, C., Rouel, J., Jouany, J. P., Doreau, V., & Chilliard, Y. (2008). Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *Journal of Animal Science, 86*, 2642–2650.

Mathieu, F., Jouany, J. P., Senaud, J., Bohatier, J., Berthin, G., & Mercier, M. (1996). The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep; protozoal and probiotic interactions. *Reproduction Nutrition Development, 36*, 271–287.

McAllister, T. A., & Newbold, C. J. (2008). Redirecting rumen fermentation to reduce methanogenesis. *Australian Journal of Experimental Agriculture, 48*, 7–13.

McCrabb, G. J., Berger, K. T., Magner, T., May, C., & Hunter R. A. (1997). Inhibiting methane production

in Brahman cattle by dietary supplementation with a novel compound and the effects on growth. *Australian Journal of Agricultural Research, 48*, 323–329.

McGinn, S. M., Beauchemin, K. A., Coates, T., & Colombatto, D. (2004). Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *Journal of Animal Science, 82*, 3346–3356.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., & Newbold, C. J. (2003). Effects of essential oils on ruminal microorganisms and their protein metabolism. *Applied and Environmental Microbiology, 69*, 5011–5014.

McMahon, L. R., Majak, W., McAllister, T. A., Hall, J. W., Jones, G. A., Popp, J. D. et al. (1999). Effect of sainfoin on in vitro digestion of fresh alfalfa and bloat in steers. *Canadian Journal of Animal Science, 79*, 203–212.

Migeotte, M. V. (1948). Methane in earth's atmosphere. *Journal of Astrophysics, 107*, 400–403.

Min, B. R., Pinchak, W. E., Fulford, J. D., & Puchala, R. (2005). Wheat pasture bloat dynamics, in vitro ruminal gas production, and potential bloat mitigation with condensed tannins. *Journal of Animal Science, 83*, 1322–1331.

Min, B. R., Pinchak, W. E., Anderson, R. C., Fulford, J. D., & Puchala, R. (2006). Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat. *Journal of Anima Science, 84*, 2546–2554.

Mitsumori, M., Ajisaka, N., Tajima, K., Kajikawa, H., & Kurihara, M. (2002). Detection of Proteobacteria from the rumen by PCR using methanotroph-specific primers. *Letters in Applied Microbiology, 35*, 251–255.

Mohammed, N., Lila, Z. A., Ajisaka, N., Hara, K., Mikuni, K., Hara, K. et al. (2004a). Inhibition of ruminal microbial methane production by beta-cyclodextrin iodopropane, malate and their combination in vitro. *Journal of Animal Physiology and Animal Nutrition, 88*, 188–195.

Mohammed, N., Lila, Z. A., Tatsuoka (Ajisaka), N., Hara, K., Mikuni, K., Hara, K. et al. (2004b). Effects of cyclodextrin-iodopropane complex on methane production, ruminal fermentation and microbes, digestibility and blood metabolites in steers. *Animal Science Journal, 75*, 131–137.

Morgavi, D. P., Jouany, J. -P., & Martin, C. (2008). Changes in methane emission and rumen fermentation parameters induced by refaunation in sheep. *Australian Journal of Experimental Agriculture, 48*, 69–72.

Moss, A. R., Jouany, J. -P., & Newbold, C. J. (2000). Methane production by ruminants: its contribution to global warming. *Annales de Zootechnie, 49*, 231–253.

Mutsvangwa, T., Edward, I. E., Topps, J. H., & Paterson, G. F. M. (1992). The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. *Animal Production, 55*, 35–40.

Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I. et al. (2004). Effects of including 1,4-galactooligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. *Animal Feed Science and Technology, 115*, 313–326.

Newbold, C. J., & Rode, L. M. (2006). Dietary additives to control methanogenesis in the rumen. In Soliva, C. R., Takahashi, J., and Kreuzer, M. (Eds.), *Greenhouse Gases and Animal Agriculture: An Update* (pp. 138–147). Amsterdam: Elsevier. International Congress Series No. 1293.

Newbold, C. J., Hassan, S. M. E., Wang, J., Ortega, M. E., & Wallace, R. J. (1997). Influence of foliage from African multipurpose tree on activity of rumen protozoa and bacteria. *British Journal of Nutrition, 78*, 237–249.

Odongo, N. E., Bagg, R., Vessie, G., Dick, P., Or-Rashid, M. M., Hook, S. E. et al. (2007a). Long-term effects of feeding monensin on methane production in lactating dairy cows. *Journal of Dairy Science, 90*, 1781–1788.

Odongo, N. E., Or-Rashid, M. M., Kebreab, E., France, J., & McBride, B. W. (2007b). Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *Journal of Dairy Science, 90*, 1851–1858.

Ohene-Adjei, S., Chaves, A. V., McAllister, T. A., Benchaar, C., Teather, R. M., & Forster, R. J. (2008). Evidence of increased diversity of methanogenic archaea with plant extract supplementation. *Microbial Ecology, 56*, 234–242.

Olivier, J. G. J., van Aardenne, J. A., Dentener, F., Ganzeveld, L., & Peters, J. A. H. W. (2005). Recent trends in global greenhouse gas emissions: Regional trends and spatial distribution of key sources. In A. van Amstel (Ed.) *Non-CO2 greenhouse gases (NCGG-4)* (pp. 325–330). Rotterdam: Millipress.

Patra, A. K., & Saxena, J. (2009). The effect and mode of action of saponins on microbial populations and fermentation in the rumen and ruminant production. *Nutrition Research Reviews, 22*, 204–219.

Patra, A. K., & Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry, 71*, 1198–1222.

Patra, A. K., Kamra, D. N., & Agarwal, N. (2006a). Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Animal Feed Science and Technology, 128*, 276–291.

Patra, A. K., Kamra, D. N., & Agarwal, N. (2006b). Effect of plants containing secondary metabolites on in vitro methanogenesis, enzyme profile and fermentation of feed with rumen liquor of buffalo. *Animal Nutrition and Feed Technology, 6*, 203–213.

Patra, A. K., Kamra, D. N., & Agarwal, N. (2008). Effect of leaf extracts on fermentation of feds and methanogenesis with rumen liquor of buffalo. *Indian Journal of Animal Science, 78*, 91–96.

Patra, A. K., Kamra, D. N., & Agarwal, N. (2010a). Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds in vitro. *Journal of the Science of Food and Agriculture, 90*, 511–520.

Patra, A. K., Kamra, D. N., Bhar, R., Kumar, R., Chaturvedi, V. B., & Agarwal, N. (2010b). Effect of *Terminalia chebula* and *Allium sativum* on nutrient utilization and methane production in sheep. *Journal of Animal Physiology and Animal Nutrition, 95*, 187–191.

Pen, B., Takaura, K., Yamaguchi, S., Asa, R., & Takahashi, J. (2007). Effects of *Yucca schidigera* and *Quillaja saponaria* with or without β-1,4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. *Animal Feed Science and Technology, 138*, 75–88.

Pinares-Patiño, C. S., Ulyatt, M. J., Lassey, K. R., Barry, T. N., & Holmes, C. W. (2003). Persistence of differences between sheep in methane emission under generous grazing conditions. *Journal of Agricultural Science, 140*, 227–233.

Puchala, R., Min, B. R., Goetsch, A. L., & Sahlu, T. (2005). The effect of a condensed tannin-containing forage on methane emission by goats. *Journal of Animal Science, 83*, 182–186.

Sahoo, B., Saraswat, M. L., Haque, N., & Khan, M. Y. (1999). Energy balance and methane production in sheep fed chemically treated wheat straw. *Small Ruminant Research, 35*, 13–19.

Santoso, B., Mwenya, B., Sar, C., Gamo, Y., Kobayashi, T., Morikawa, R. et al. (2004). Effects of supplementing galacto-oligosaccharides, *Yucca schidigera* and nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. *Livestock Production Science, 91*, 209–217.

Sar, C., Santoso, B., Gamo, Y., Kobayashi, T., Shiozaki, S., Kimura, K. et al. (2004a). Effects of combination of nitrate with β-1,4 galacto-oligosaccharides and yeast (*Candida kefyr*) on methane emission from sheep. *Asian-Australasian Journal of Animal Sciences, 17*, 73–79.

Sar, C., Santoso, B., Mwenya, B., Gamo, Y., Kobayashi, T., Morikawa, R. et al. (2004b). Manipulation of rumen methanogenesis by the combination of nitrate with β-1,4 galacto-oligosaccharides or nisin in sheep. *Animal Feed Science and Technology, 115*, 129–142.

Sar, C., Mwenya, B., Pen, B., Morikawa, R., Takaura, K., Kobayashi, T. et al. (2005). Effect of nisin on ruminal methane production and nitrate/nitrite reduction in vitro. *Australian Journal of Agricultural Research, 56*, 803–810.

Sauer, F. D., Fellner, V., Kinsman, R., Kramer, J. K., Jackson, H. A., Lee, A. J. et al. (1998). Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. *Journal of Animal Science, 76*, 906–914.

Singh, G. P. (2001). Livestock production and environmental protection. In *Proceedings of the 10th Animal Nutrition Conference* (pp. 211–221), National Dairy Research Institute, Karnal, Haryana, India.

Sirohi, S., Michaelowa, A., & Sirohi, S. K. (2007). Mitigation options for enteric methane emissions from dairy animals: An evaluation for potential CDM project in India. *Mitigation and Adaptation Strategies for Global Change, 12*, 259–274.

Sliwinski, B. J., Solvia, C. R., Machmuller, A., Kreuzer, M. (2002). Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. *Animal Feed Science and Technology, 101*, 101–114.

Srivastava, A. K., & Garg, M. R. (2002). Use of sulfur hexafluoride tracer technique for measurement of methane emission from ruminants. *Indian Journal of Dairy Science, 55*, 36–39.

Steinfeld, H., Costales, A., Rushton, J., Scherf, B., Bennett, T., & Hall, D. (2006). *Livestock report 2006*. Rome: FAO.

Thornton, J. H., & Owens, F. N. (1981). Monensin supplementation and in vivo methane production by steers. *Journal of Animal Science, 52*, 628–634.

Trei, J. E., Parish, R. C., Singh, Y. K., & Scott G. C. (1972). Effect of methane inhibitors on rumen metabolism and feedlot performance of sheep. *Journal of Dairy Science, 54*, 536–540.

Ulyatt, M. J., Lassey, K. R., Shelton, I. D., & Walker, C. F. (2002). Methane emission from dairy cows and wether sheep fed subtropical grass-dominant pastures in midsummer in New Zealand. *New Zealand Journal of Agricultural Research, 45*, 227–234.

Ungerfeld, E. M., Kohn, R. A., Wallace, R. J., & Newbold, C. J. (2007). A meta-analysis of fumarate effects on methane production in ruminal batch cultures. *Journal of Animal Science, 85*, 2556–2563.

Van Nevel, C. J., & Demeyer, D. I. (1996). Control of rumen methanogenesis. *Environmental Monitoring and Assessment, 42*, 73–97.

Van Vugt, S. J., Waghorn, G. C., Clark, D. A., & Woodward, S. L. (2005). Impact of monensin on methane production and performance of cows fed forage diets. *Proceedings of the New Zealand Society of Animal Production, 65*, 362–366.

Waghorn, G. C., Tavendale, M. H., & Woodfield, D. R. (2002). Methanogenesis from forages to sheep. *Proceedings of the New Zealand Society of Grassland Association, 64*, 167–171.

Waghorn, G. C., Clark, H., Taufa, V., & Cavannagh, A. (2007). Monensin controlled release capsules for improved production and mitigating methane in dairy cows fed pasture. *Proceedings of the New Zealand Society of Animal Production, 67*, 266–271.

Wallace, R. J., Wood, T. A., Rowe, A., Price, J., Yanez, D. R., Williams, S. P. et al. (2006). Encapsulated fumaric acid as a means of decreasing ruminal methane emissions. *International Congress Series, 1293*, 148–151.

Wang, C. J., Wang, S. P., & Zhou, H. (2009). Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. *Animal Feed Science and Technology, 148*, 157–166.

Wedegaertner, T. C., & Johnson, D. E. (1983). Monensin effects on digestibility, methanogenesis and heat increment of a cracked corn-silage diet fed to steers. *Journal of Animal Science, 57*, 168–177.

Wedlock, D. N., Pedersen, G., Denis, M., Dey, D., Janssen, P. H., & Buddle, B. M. (2010). Development of a vaccine to mitigate greenhouse gas emissions in agricul-

ture: vaccination of sheep with methanogen fractions induces antibodies that block methane production in vitro. *New Zealand Veterinary Journal, 58*, 29–36.

Weimer, P. J. (1998). Manipulating ruminal fermentation: a microbial ecological perspective. *Journal of Animal Science, 76*, 3114–3122.

Williams, Y. J., Rea, S. M., Popovski, S., Pimm, C. L., Williams, A. J., Toovey, A. F. et al. (2008). Responses of sheep to a vaccination of entodinal or mixed rumen protozoal antigens to reduce rumen protozoal numbers. *British Journal of Nutrition, 99*, 100–109.

Wina, E., Muetzel, S., Hoffmann, E., Makkar, H. P. S., & Becker, K. (2005). Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure in vitro. *Animal Feed Science and Technology, 121*, 159–174.

Woodward, S. L., Waghorn, G. C., Ulyatt, M. J., & Lassey, K. R. (2001). Early indication that feeding lotus will reduce methane emission from ruminants. *Proceedings of the New Zealand Society of Animal Production, 61*, 23–26.

Woodward, S. L., Waghorn, G. C., Lassey, K. R., & Laboyrie, P. G. (2002). Does feeding sulla (*Hedysarum coronarium*) reduce methane emission from dairy cows? *Proceedings of the New Zealand Society of Animal Production, 62*, 227–230.

Wright, A. -D. G., Kennedy, P., O'Neill, C. J., Toovey, A. F., Popovski, S., Rea, S. M. et al. (2004). Reducing methane emission in sheep by immunization against rumen methanogens. *Vaccine, 22*, 3976–3985.

Yuan, Z. P., Zhang, C. M., Zhou, L., Zou, C. X., Guo, Y. Q., Li, W. T., et al. (2007). Inhibition of methanogenesis by tea saponin and tea saponin plus disodium fumarate in sheep. *Journal of Animal and Feed Sciences, 16*(Supplement 2), 560–565.

Zhou, M., Hernandez-Sanabria, E., & Guan, L. L. (2009). Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. *Applied and Environmental Microbiology, 75*, 6524–6533.