



Abatement of Methane Production from Ruminants: Trends in the Manipulation of Rumen Fermentation*

Yasuo Kobayashi**

Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

ABSTRACT : Methane emitted from ruminant livestock is regarded as a loss of feed energy and also a contributor to global warming. Methane is synthesized in the rumen as one of the hydrogen sink products that are unavoidable for efficient succession of anaerobic microbial fermentation. Various attempts have been made to reduce methane emission, mainly through rumen microbial manipulation, by the use of agents including chemicals, antibiotics and natural products such as oils, fatty acids and plant extracts. A newer approach is the development of vaccines against methanogenic bacteria. While ionophore antibiotics have been widely used due to their efficacy and affordable prices, the use of alternative natural materials is becoming more attractive due to health concerns regarding antibiotics. An important feature of a natural material that constitutes a possible alternative methane inhibitor is that the material does not reduce feed intake or digestibility but does enhance propionate that is the major hydrogen sink alternative to methane. Some implications of these approaches, as well as an introduction to antibiotic-alternative natural materials and novel approaches, are provided. (**Key Words :** Rumen, Methane, Microbes, Fermentation, Hydrogen Sinks)

INTRODUCTION

Rumen microbes consisting of protozoa, fungi and bacteria play a pivotal role in rumen fermentation including fiber digestion. Fermentation results in the synthesis of various products, some of which are not entirely beneficial. One such non-beneficial product is methane. This gaseous compound is the most prominent hydrogen sink product synthesized in the rumen. Since methane contains energy, its emission during rumen fermentation is considered to be a loss of feed energy that is equivalent to 2-12% of the gross energy of animal feed (Johnson and Johnson, 1995).

Another negative aspect of methane emission from ruminants is its possible contribution to global warming. Annual methane production from cattle is responsible for 15-20% of global methane production. This level of production corresponds to ca. 3-5% of global CO₂ production when converted to CO₂ based on the global warming effect of methane (23x CO₂) (IPCC, 2001).

Scientists have been trying to reduce methane emission

from ruminants since the 1950s with the aim of saving feed energy. Although some manipulations have been successful, their use has been limited due to environmental and human health concerns. Indeed, ionophore antibiotics such as monensin, which are effective in reducing rumen methanogenesis to some extent, have not been available in the EU since 2006 because of these concerns. In the meantime, alternative bactericidal compounds originating from natural products, mainly from plants, have been explored and some are available on a commercial basis, although their reported efficacy is variable (Wina et al., 2005; Calsamiglia et al., 2007). This mini-review describes recent progress in research on abatement of methane production by ruminants from the aspect of rumen microbial manipulation.

HYDROGEN SINKS IN THE RUMEN

The rumen is an anaerobic fermentation chamber, in which diverse and dense microbial populations have symbiotic relationships in which metabolites are exchanged that promote or compensate each others growth, a process which is termed "cross feeding" (Wolin et al., 1997). Methane synthesis is regarded as one such cross feeding between hydrogen-producing microbes and hydrogen-consuming methanogens. Since the hydrogen-producing

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** Corresponding Author: Yasuo Kobayashi. Tel & Fax: +81-11-706-2476, E-mail: kyas@anim.agr.hokudai.ac.jp

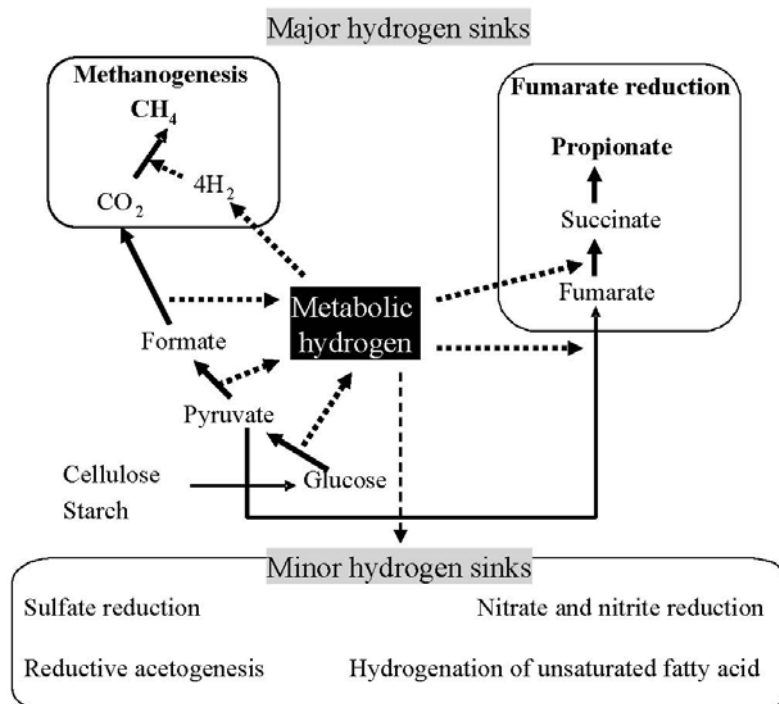


Figure 1. Hydrogen-consuming pathways recognized in the rumen.

microbes include fibrolytic fungi and bacteria, their co-association with methanogens allows efficient removal of hydrogen, which facilitates continuous fiber degradation.

When methane reduction is attempted, it is therefore necessary to consider alternative hydrogen sinks to methanogenesis. Figure 1 shows the hydrogen-consuming pathways currently known to exist in the rumen. Methanogenesis is the primary pathway followed by propionate production (fumarate reduction) (Mitsumori and Sung, 2008). Other reactions (nitrate- and nitrite reduction, reductive acetogenesis, biohydrogenation of unsaturated fatty acid) play a relatively minor role in hydrogen consumption within the rumen. Thus, it is reasonable that a strategy for methane mitigation is developed concomitantly with a strategy to enhance propionate production. Otherwise, rumen fermentation could be hindered by hydrogen accumulation caused by the lack of hydrogen removal by methanogenesis. Accordingly, propionate enhancement could be a good indicator of simultaneous methane reduction in the rumen. A number of studies on methane reduction have been performed along these lines and, indeed, rumen microbial numbers and their metabolic activities do change with methane reduction. However, the manner of these changes varies depending on the manipulation procedure, *i.e.* whether chemicals and vaccines directly active against methanogens, or antibiotics and plant-derived antimicrobial compounds that indirectly affect methanogenesis, are used.

HALOGENS AND OTHER METHANE-INHIBITING CHEMICALS

Several anti-methanogenic compounds have been documented since the 1970s (Chalupa et al., 1977). Although all these compounds are effective in the reduction of methane production, individual compounds have specific disadvantages which inhibit their current practical use. Thus some compounds are regarded as ozone disruptive agents, while others are expensive or have safety concerns. However, these compounds are good models for the study of shifts in rumen fermentation and microbiota that occur when methanogens and methane production are drastically inhibited.

Ungerfeld et al. (2004) evaluated the sensitivity of representative rumen methanogens to potent methanogen inhibitors including 2-bromoethanesulphonate (BES), 3-bromopropanesulphonate (BPS), limazine, propynoic acid and ethyl 2-butynoate. All of these chemicals, with the exception of BPS, inhibited methane production from *Methanobrevibacter ruminantium*, *Methanosarcina mazei* and *Methanomicrobium mobile*. The sensitivity of the methanogens to each chemical was species-dependent, suggesting that sensitive methanogens can be replaced by resistant methanogens following administration of the chemical over a certain period of time.

Bromochloromethane (BCM) inhibits cobamide-dependent methanogenesis in which the majority of rumen methanogens are involved. When BCM was fed to cattle,

the total methane emission was reduced by ca. 30% with a resultant increase in propionate and branched chain fatty acids as alternative hydrogen sinks (Denman et al., 2007). These changes were accompanied by an average decrease of 34% in the number of methanogens quantified by *mcrA*-targeted real-time PCR. BCM feeding led to diversification of the methanogen population even though the total population size was decreased. This result implies that alternative methanogens (*Methanomicrobium*, *Methanosarcina*, *Methanococcus* and unknown methanogens) are developed following the suppression of major methanogens such as *Methanobacterium* by BCM. Thus, a change in the methanogen population diversity in response to an inhibitor should be considered as a possible consequence of the manipulation of methanogens via the mitigation strategy of chemical intervention.

VACCINES AGAINST METHANOGENS

A unique attempt to reduce rumen methane is ongoing in Australia. This approach does not involve dietary manipulation by the inclusion of additives but involves vaccination of the animal against methanogenic bacteria in the rumen. Wright et al. (2004) reported that a significant 7.7% reduction in rumen methane production, corrected for dry matter intake level, was achieved by this immunization strategy. They estimated that less than 20% of the methanogens were targeted by the vaccine that was prepared using 3 *Methanobrevibacter* strains. A vaccine of broader range is being developed to induce a greater extent of methane reduction. One such vaccine (targeting >52% of different species/strains of methanogens that were tested based on a survey of sheep prior to vaccination) was applied to 32 sheep. Although the animals showed specific IgG titers in plasma, saliva and rumen fluid, neither methane output nor the number of rumen methanogens was significantly changed (Williams et al., 2009). The development of alternative methanogens after immunization is a possible reason for this failure and a much more broad-spectrum approach together with a more comprehensive understanding of the rumen methanogen population is surely required for the vaccination approach to be successful.

BACTERIOCINS

Naturally occurring anti-bacterial agents, bacteriocins, originating from rumen bacteria have been reviewed by Teather and Forster (1998) who pointed out their possible use as modifiers of rumen fermentation. These bacteriocins may also be useful for the prevention of animal metabolic disorders such as lactic acidosis and bloat and may even prove useful for food storage.

Bovicin, a bacteriocin produced by *Streptococcus bovis*, has been reported as a possible methane-mitigating agent in the rumen (Lee et al., 2002). Supplementation of a mixed rumen bacterial culture with bovicin inhibited methane production by as much as 53%. When the culture was transferred successively (50% v/v) with bovicin, it lost the ability to produce methane after only 5 transfers. Moreover, the restriction pattern of amplified 16S archaeal rDNA was not different between cultures with and without bovicin indicating that the effect of bovicin on rumen methanogens might not be selective. Activity of bovicin against other rumen bacteria or an effect of bovicin on the fermentation pattern has not been reported and remains to be investigated.

The first described bacteriocin, nisin, that is produced by *Lactococcus lactis*, also has a methane-mitigating ability that was observed in a monensin-supplemented *in vitro* culture (ca. 20% inhibition without a negative effect on volatile fatty acid (VFA) production) (Callaway et al., 1997). Although no mechanism was proposed to explain its effect on rumen bacteria, nisin does potentiate propionate production and possibly shows selective activity against Gram-positive rumen bacteria.

The above bacteriocins are known to be as potent as monensin and are also active even at low pH. However, further investigation is necessary before they can be considered as candidate additives for ruminant livestock, in particular for beef cattle fed a high grain diet.

IONOPHORE ANTIBIOTICS

Ionophore antibiotics, represented by monensin, have been widely used all over the world as feed additives for ruminant livestock since the mid 1970s. Monensin is considered as a growth promoter due to its favorable effects on rumen fermentation including methane reduction, propionate enhancement and ammonia reduction, together with its preventive effects on coccidiosis, bloat and lactic acidosis. These effects are attributed to a selective antimicrobial action of monensin on rumen microbes. Monensin is inhibitory for protozoa, Gram-positive bacteria including ruminococci, streptococci and lactobacilli but not for Gram negative bacteria, and therefore leads to rumen microbiota that produce more propionate and less acetate, butyrate, formate and hydrogen (Russell and Strobel, 1989). Partial inhibition of hydrogen- and formate-producing microbes contributes to methane reduction, the extent of which varies between reports. Review papers indicate that methane reduction by monensin ranges from 4 to 31% (Schelling et al., 1984; Rumpler et al., 1986). A recent report indicated that long term administration of monensin to dairy cattle stably reduced methane by 7% and that this reduction persisted for 6 months with no adverse effect on milk yield (Odongo et al., 2007). However, beef steers that

had been given monensin only showed methane reduction during the first 4-6 weeks of administration. Nevertheless, it should be noted that propionate enhancement persisted throughout the 14 wk experiment (Guan et al., 2006).

The number of rumen protozoa is decreased by ionophores and this decrease causes a reduction in methane, because rumen protozoa accommodate methanogens on their cell surface and within the cell (Vogels et al., 1980; Tokura et al., 1999). Therefore the number and/or activity of methanogens are believed to be indirectly reduced by ionophores. This is part of the reason why methane reduction by ionophores occurs only at the early stage of feeding since rumen protozoan populations that are depressed by ionophores tend to restore their numbers when ionophores are administered for a long time (Kobayashi et al., 1988).

However, long term feeding of monensin does not affect the number of methanogens (Hook et al., 2009). These data suggest that reduction of methane by monensin feeding is not due to a reduction in the population size of methanogens but is more likely due to the development of an alternative hydrogen-consuming pathway such as propionate enhancement by stimulation of the proliferation of propionate- and succinate-producing bacteria such as *Selenomonas* and *Megasphaera* (Russell and Strobel, 1989).

Such rumen bacterial selection by monensin appears to be maintained even months following administration which probably explains the persistence of propionate enhancement. Although the effect of monensin on rumen fiber digestion is inconsistent, one of the most dominant fibrolytic bacteria, *Fibrobacter succinogenes*, appears to be insensitive to monensin as its abundance within the rumen, monitored by a DNA probing method, was not affected by monensin (Stahl et al., 1988).

OILS AND FATTY ACIDS

Plant oils rich in medium chain fatty acids are known to inhibit rumen methanogenesis. One such oil, coconut oil, is particularly effective (Dohme et al., 2000). The major component of coconut oil is lauric acid (C14:0) which is more potent in the reduction of methane in a semi-continuous fermenter that simulates the rumen (RUSITEC) than other fatty acids including palmitic (C16:0), stearic (C18:0) and linoleic (C18:2) acids (Dohme et al., 2001). A similar reduction in methane was observed in batch cultures, in which coconut oil and lauric acid were directly compared, and which showed that lauric acid inhibited methanogenesis to a greater extent (Yabuuchi et al., 2006, 2007).

Lauric acid is inhibitory for Gram-positive rumen bacteria including cellulolytic ruminococci. Therefore, addition of lauric acid to feed might decrease feed

digestibility of a high roughage diet. However, a decrease in feed digestibility would be negligible with the high concentrate diet that is fed to beef cattle. Lauric acid was also shown to depress the metabolic activity of the saccharolytic bacterium *Streptococcus bovis* without affecting its maximal growth. The decreased lactate production by *S. bovis* in the presence of lauric acid may explain the preventive and curing effects of lauric acid on rumen lactic acidosis. These data suggest that lauric acid may not alter the size of a specific bacterial population but may modulate metabolic activity when it is fed over a long period of time. Indeed, the abundance of other rumen bacterial species was not altered following lauric acid feeding (Yabuuchi et al., 2007).

Most of the oils and fatty acids that reduce methanogenesis reduce the ruminal level of protozoa that are known to be cosymbionts of methanogens as mentioned in the section on ionophores. Therefore, a reduction in protozoan numbers is partly responsible for the decreased methane production induced by oils and fatty acids.

PLANTS AND THEIR EXTRACTS

Many candidate feed additives originating from plant materials have been screened for their potential ability to reduce rumen methanogenesis. One such compound is saponin. Although the inhibitory effect of saponin and sarsaponin on methanogenesis varies with the plant source, inhibition ranging from approximately 5-60%, accompanied by enhanced propionate, has been reported (Wina et al., 2005). Saponins have a detergent action that disrupts microbial cell membranes by formation of a complex with membrane sterols. Rumen protozoa are particularly sensitive to saponins which reduce their level in the rumen, resulting in the depression of methanogens associated with protozoa. Guo et al. (2007) have suggested that a decrease in methanogens associated with protozoa as exo- and endo-symbionts could be the main mechanism by which saponin feeding reduces methanogenesis.

Some essential oils that possess antibacterial activity are commercially available. The main components of essential oils that exert antibacterial activity are considered to be a variety of compounds that are mainly classified as terpenoids or phenylpropanoids. Their antibacterial spectra are relatively broad and their mechanism of action involves interaction of the antibacterial compound with the bacterial cell membrane which destabilizes the membrane. Although favorable depressive effects of essential oils on rumen proteolysis and deamination have been demonstrated, reports of their potency for the reduction of rumen methanogenesis are inconsistent (Calsamiglia et al., 2007).

European scientists have been collaborating in an

exploration of plants that might be useful as alternatives to antibiotics for inhibition of methanogenesis in ruminant livestock. This project has been termed "RUMEN-UP". (http://www.rowett.ac.uk/rumen_up/index.html). Seven potential candidates were ultimately selected from 500 different plant species based on their ability to inhibit methane production by 15-27% without a detrimental effect on total VFA production or feed digestibility. The application of these candidates to ruminant livestock is still at the beginning stage and many points still need to be clarified. The plant species selected were the Italian plumeless thistle (*Carduus pycnocephalus*, 30% inhibition), the Chinese peony (*Paeonia lactiflora*, 8-53%), the European aspen (*Populus tremula*, 25%), the sweet cherry (*Prunus avium*, 20%), goat willow (*Salix caprea*, 30%), English oak (*Quercus pedunculata*, 25%) and Sikkim rhubarb (*Rheum nobile*, 25%) (Table 1). From these species, a final 2 species (*Carduus* and *Rheum*) were evaluated as to their potency in a RUSITEC analysis. On a high forage diet 16 and 22% inhibition of methanogenesis respectively was noted, while less inhibition (5 and 15% respectively, not significant) was observed on a high concentrate diet. Methane reduction was not accompanied by propionate enhancement or other favorable fermentation changes. No clear dose response was observed and solvent extraction diminished the inhibitory effect. Therefore, details of the inhibition, such as the identity of the effective compound and its mechanism of action, remain to be clarified. While RUMENUP was successful in the exploration of antiproteolytic plant species that have already been confirmed *in vivo*, it is still inconclusive whether any plant species is useful for the reduction of methane production from the rumen.

NEW MATERIALS

Recent research in Japan has revealed two potential natural materials for the reduction of rumen methanogenesis; plant-derived liquid (PDL) and yeast-derived surfactant (YDS). Both of these materials have induced a dramatic reduction in methane production in batch cultures (>95%) and in RUSITEC (>70%) without any adverse effect on feed digestibility or total VFA

production. The extent of inhibition induced by these new materials is much greater than that induced by monensin or by the materials proposed in RUMENUP.

PDL contains anacardic acid, a salicylic acid derivative with an alkyl group that inhibits Gram-positive bacteria including bacilli and staphylococci (Kubo et al., 1993). Therefore, PDL is expected to selectively inhibit Gram-positive rumen bacteria. Anacardic acid was suggested to be a propionate enhancer in early studies (Van Nevel et al., 1971), although this fact has not been highlighted for a long time. The surfactant YDS disrupts bacterial cell walls in a selective manner depending on the structure of the bacterial surface. Gram-negative bacteria possess an outer membrane that minimizes bacterial cell damage from such a surfactant. Thus, YDS might also selectively inhibit Gram positive rumen bacteria. Indeed, YDS and PDL showed a similar antibacterial spectrum when tested against 13 representative rumen bacterial species. Propionate and succinate producers such as *Selenomonas ruminantium*, *Megasphaera elsedenii*, and *Succinivibrio dextrinosolvens* were tolerant to these two materials, while hydrogen and formate producers such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, and *Eubacterium ruminantium* were sensitive. Therefore, both of these materials are believed to shift rumen fermentation toward more propionate and less methane production through selective anti-bacterial activities.

The anti-bacterial selectivity of these materials that was indicated in the above pure culture study was fairly well reflected when the bacteria were analyzed in RUSITEC, in which the DGGE banding pattern was apparently changed following supplementation with either material. Thus, the abundance of *M.elsedenii* and *S. dextrinosolvens* increased as estimated by real-time PCR assays (Table 2) and as judged by their detection frequency in clone library analyses. A more favorable fermentation pattern was observed following addition of the materials that was shifted, as expected, towards more propionate and less methane and there were no changes in total VFA production or feed digestibility. Sheep that were fed a diet supplemented with PDL or YDS showed a fermentation pattern that was similar to that observed in RUSITEC and

Table 1. Selected plants potentially inhibiting rumen methanogenesis in the RUMEN-UP project

Common name	Duration	Distribution	CH ₄ inhibition (%)
Italian plumeless thistle	Annual	World-wide (temperate parts)	30
Chinese peony	Perennial	China	8-53
European aspen	Perennial	Europe	25
Sweet cherry	Perennial	Europe, western Asia and north Africa	20
Goat willow	Perennial	Europe, north east Africa and central Asia	30
English oak	Perennial	Europe, western Asia and northern Africa	25
Sikkim rhubarb	Perennial	Himalayas	25

Table 2. Effect of plant-derived liquid (PDL) on the abundance of representative rumen bacteria in RUSITEC expressed as the log of 16S rDNA copy number/ml determined by real-time PCR

	Control	PDL	Effect of PDL
Total bacteria	9.31	9.39	none
Total methanogen	7.52	7.58	none
Hydrogen and formate producer			
<i>Ruminococcus flavefaciens</i>	6.55	5.40*	negative
<i>Ruminococcus albus</i>	7.41	7.38	none
<i>Treponema bryantii</i>	4.37	2.42*	negative
Lactate producer			
<i>Streptococcus bovis</i>	3.59	3.34	none
Succinate and propionate producer			
<i>Fibrobacter succinogenes</i>	7.40	5.86*	negative
<i>Prevotella ruminicola</i>	7.95	7.28*	negative
<i>Prevotella bryantii</i>	6.56	5.38*	negative
<i>Succinimonas dextrinosolvens</i>	6.96	7.61*	positive
<i>Ruminobacter amylophilus</i>	3.29	4.20*	positive
<i>Anaerovibrio lipolytica</i>	7.20	8.44*	positive
<i>Selenomonas ruminantium</i>	5.41	5.74*	positive
<i>Megasphaera elsdenii</i>	7.32	8.39*	positive

* Significantly different from control value

was accompanied by similar bacterial population shifts. Further evaluation of these materials as additives is currently being conducted in cattle.

CONCLUSIONS

Stimulation of propionate production could be the best alternative hydrogen sink to methanogenesis in the rumen. Therefore, a strategy for abatement of methane production should be considered concurrently with a strategy to enhance propionate production. Although various feed additive candidates are now available to achieve this aim, the choice of additive must depend on the potency, safety, and expense of the candidate additive.

Since our understanding of rumen microbes is still incomplete, elucidation of microbial diversity and microbial interrelationships is absolutely essential for the successful manipulation of rumen fermentation towards a significant reduction in ruminant methane emission. Attainment of such knowledge would permit the realization of abatement of rumen methane production in a more successful manner than heretofore.

New approaches for methane reduction such as vaccination of ruminants against methanogens and the application of wallaby foregut microbiota that produce much less methane than the microbiota of cattle rumen (Morrison et al., 2008), are still at a fundamental stage of development. However, the backbone of the future success of these approaches is also a comprehensive analysis of

microbiota and a systematic understanding of their biological function.

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