



## Control of Rumen Microbial Fermentation for Mitigating Methane Emissions from the Rumen\*

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**ABSTRACT :** The rumen microbial ecosystem produces methane as a result of anaerobic fermentation. Methanogenesis in the rumen is thought to represent a 2-12% loss of energy intake and is estimated to be about 15% of total atmospheric methane emissions. While methanogenesis in the rumen is conducted by methanogens, PCR-based techniques have recently detected many uncultured methanogens which have a broader phylogenetic range than cultured strains isolated from the rumen. Strategies for reduction of methane emissions from the rumen have been proposed. These include 1) control of components in feed, 2) application of feed additives and 3) biological control of rumen fermentation. In any case, although it could be possible that repression of hydrogen-producing reactions leads to abatement of methane production, repression of hydrogen-producing reactions means repression of the activity of rumen fermentation and leads to restrained digestibility of carbohydrates and suppression of microbial growth. Thus, in order to reduce the flow of hydrogen into methane production, hydrogen should be diverted into propionate production via lactate or fumarate. (**Key Words :** Methane, Rumen, Rumen Microorganisms, Methanogens)

### INTRODUCTION

Rumen microbial fermentation supplies host animals (ruminants) with volatile fatty acids (VFAs) and microbial proteins as fermentation products. In the mean time, rumen microbial fermentation also release methane as a fermentation product into the atmosphere. It has been estimated that methane production by ruminants is about 15% of total atmospheric methane emissions (Takahashi et al., 2005). Furthermore, methanogenesis in the rumen is thought to represent a 2-12% energy loss of intake (Czerkawski, 1969). Rumen microbial fermentation is conducted by the rumen microbial ecosystem, in which many kinds of microorganisms, such as rumen bacteria, protozoa and fungi, anaerobically convert their substrates into fermentation products. Then, some of them are utilized by other microorganisms for their growth. In the case of

methanogenesis, methanogens in the rumen mainly convert carbon dioxide into methane by reduction with hydrogen. Thus, methanogens may play an important role as hydrogen scavengers in the rumen and decrease hydrogen, which would suppress rumen digestion (Wolin et al., 1997), from the rumen. Here, we try to overview how to mitigate methane emission from the rumen by means of control of rumen fermentation.

### METHANE PRODUCTION IN THE RUMEN

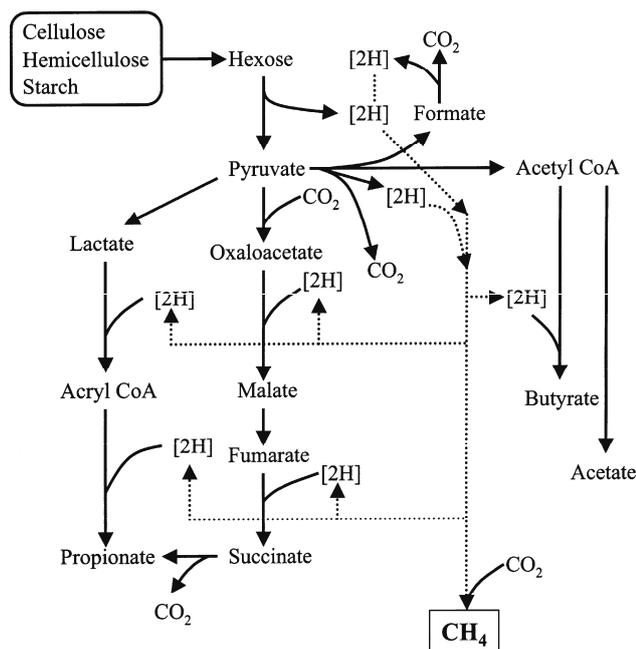
#### General metabolic pathways in the rumen

The rumen can be thought of as a kind of anaerobic fermentation tank, in which many living microorganisms, rumen microorganisms, affect each other. Nutritional components such as carbohydrates, proteins and lipids in feedstuffs are degraded by rumen microorganisms and are converted into microbial cells, which include proteins and carbohydrates, and volatile fatty acids (VFAs) and gasses. Because hydrogen derives mainly from carbohydrates and is supplied for methane production in the rumen (Figure 1), carbohydrate degradation is often the focus of efforts to abate methane production from livestock rumens.

Various carbohydrates contained in feedstuffs are

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**Figure 1.** Possible fermentation pathways of methane production in the rumen.

degraded by rumen fermentation, which is carried out by a consortium of microorganisms, i.e., the rumen microbial ecosystem. Although rumen bacteria are abundant and it is rumen bacteria that mainly support rumen fermentation, rumen protozoa and rumen fungi, which are anaerobic eukaryotes, also contribute to rumen fermentation. Fermentation pathways relating to carbohydrate utilization in the rumen have been intensively investigated; the results of such studies have shown that the major products of rumen fermentation are volatile fatty acids (VFAs), carbon dioxide and methane (Russell and Wallace, 1997). Predominant VFAs from the rumen include acetate (Ac), propionate (Pr) and butyrate (Bu). The typical stoichiometry was proposed by Wolin (1979):

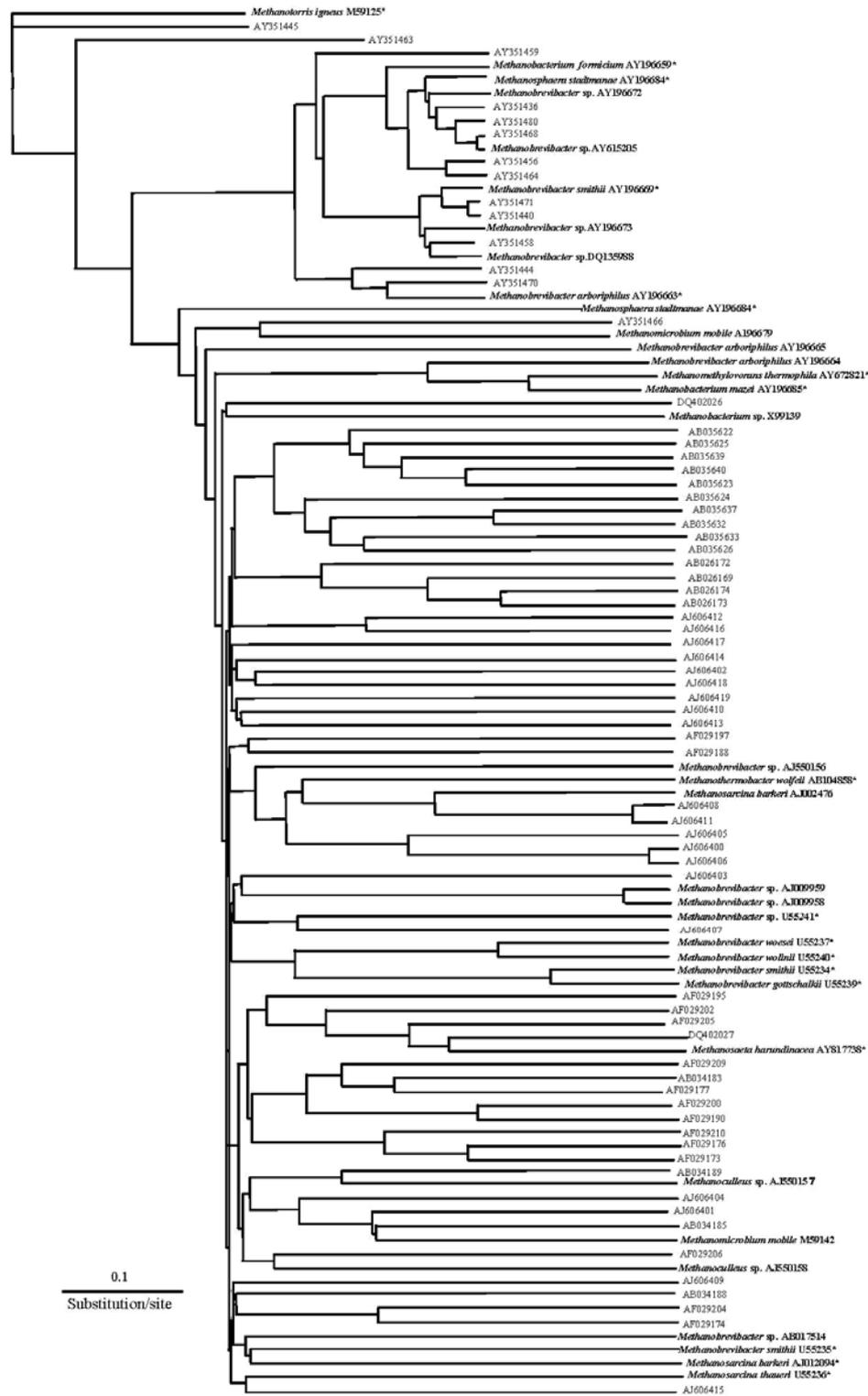


Although equation 1 is briefly formulated by an input (hexose) and outputs (VFAs, carbon dioxide, methane and water), possible fermentation pathways are shown in Figure 1. While rumen fermentation is generally performed by the rumen microbial ecosystem, individual rumen microorganisms degrade specific substrates for their growth. The rumen microorganisms eventually release into the rumen their final products of metabolism, or fermentation products, some of which are utilized by other microorganisms. For example, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* can

degrade cellulose and are thus referred to as cellulolytic bacteria. *F. succinogenes* and *R. flavefaciens* produce acetate and succinate as major fermentation products; *R. albus* produces only acetate. Succinate, produced by *Prevotella ruminicola*, *Ruminobacter amylophilus*, *F. succinogenes*, *R. flavefaciens*, *Succinivibrio dextrinosolvens*, *Succinomonas amylolytica* and other bacteria, is continuously converted to propionate and  $\text{CO}_2$  by *Selenomonas ruminantium*, for which succinate is a propionate-generating pathway intermediate, and *Veillonella alcalescens* and *Succiniclasticum ruminis* and others, which decarboxylate succinate to produce propionate (Wolin et al., 1997). Therefore, rumen fermentation is properly expressed as the total of metabolisms of individual microorganisms inhabiting the rumen.

### Methane-producing pathways in the rumen

The carbohydrate-fermenting bacteria and protozoa in the rumen produce  $\text{CO}_2$ ,  $\text{H}_2$ , and VFAs. It is known that  $\text{CO}_2$  and  $\text{H}_2$  are major precursors of  $\text{CH}_4$ ; formate is also a precursor of  $\text{CH}_4$  (Figure 1). Methane production from formate is estimated to comprise approximately 15-20% of the total methane production in the rumen (Hungate et al., 1970; Asanuma et al., 1999). The precursors for methane production are converted into  $\text{CH}_4$  by methane-producing Archaea, methanogens that appeared on Earth some  $3.5 \times 10^9$  years ago or earlier (Ueno et al., 2006). *Methanobrevibacter ruminantium*, *Methanomicrobium mobile*, *Methanosarcina mazei*, *Methanosarcina barkeri* and *Methanobacterium formicicum* have been isolated from the rumen by cultivation (Mitsumori et al., 2002). Biochemical studies of culturable methanogens have shown that *M. ruminantium*, *M. formicicum* and *M. mobile* utilize  $\text{H}_2/\text{CO}_2$  and formate to produce methane. On the other hand, *M. mazei* synthesizes methane from acetate, methanol and methylamines. *M. barkeri* utilizes  $\text{H}_2/\text{CO}_2$ , acetate, methanol and methylamines for methane synthesis (Jarvis et al., 2000). It is assumed that methyl coenzyme-M reductase (MCR) is common in the methanogens, because the final step of methane production by the methanogens is catalyzed by MCR (Ermler et al., 1997). Moreover, since methanogens contain the fluorescent compound F420 (coenzyme 420), direct observation by fluorescent microscopy has been possible, revealing the interaction between rumen ciliates and methanogens, which attach themselves to the ciliate cell surface and receive hydrogen from it (Vogels et al., 1980). Polymerase chain reaction (PCR)-based techniques have been developed for detecting methanogens from the rumen without cultivation. The small-subunit (SSU) rRNA gene (16S rDNA) types are most useful for this purpose (Wright et al., 2004). Primers targeting 16S rDNA for detecting methanogens from the rumen have been developing



**Figure 2.** Phylogenetic tree constructed with 16S rDNA sequences of rumen methanogens in the Genbank aligned by neighbor-joining method using a software, ClustalW (version 1.83. XP), with reference sequences from the Genbank. Clones are exhibited by the GenBank accession numbers. *Methanoterris igneus* (AY351437) is used as the outgroup for rooting the tree. The asterisk (\*) indicates that the sequence was not isolated from the rumen. Sequences shown by boldface indicate that the sequences were obtained from cultured strains.



**Figure 3.** Phylogenetic tree constructed with *mcrA* gene sequences of rumen methanogens in the Genbank aligned by neighbor-joining method using a software, ClustalW (version 1.83. XP), with reference sequences from the Genbank. Clones are exhibited by the GenBank accession numbers. *Methanobacterium formicicum* (AF414050) is used as the outgroup for rooting the tree. The asterisk (\*) indicates that the sequence was not isolated from the rumen. Sequences shown by boldface indicate that the sequences were obtained from cultured strains.

(Skillman et al., 2006). Uncultured methanogenic clones encoding 16S rDNA are illustrated in Figure 2, in which 16S rDNA sequences from cultured strains are also exhibited. This figure shows that a broad range of uncultured methanogens was indeed detected from the rumen. However, the biochemical properties of these uncultured methanogens in the rumen have until now been unknown.

The symbiotic relationship between ciliate and methanogens has been shown by a PCR-based technique (Tokura et al., 1999; Regensbogenova et al., 2004) as well as microscopic observations (Vogels et al., 1980). Moreover, it was demonstrated that methanogens associated with rumen ciliate not only attach to the cell surface of ciliate but also distribute themselves in the ciliate cell as endosymbionts (Finlay et al., 1994; Irbis and Ushida, 2004).

The gene encoding the  $\alpha$  subunit of the methyl-coenzyme M reductase (MCR), which catalyzes the last step in methanogenesis, is present in all methanogens (Friedrich, 2005). Thus, this gene (*mcrA*) was applied for phylogenetic analysis of methanogens like 16S rDNA genes (Luton et al., 2002). The *mcrA* genes isolated from the rumen have been used for the same purpose (Tatsuoka et al., 2004). Phylogenetic analyses of rumen *mcrA* genes registered in GeneBank are shown in Figure 3. Recently, a quantitative PCR technique was developed for detecting and quantifying methanogens in the rumen using primers targeting *mcrA* genes (Denman et al., 2006).

### STRATEGIES FOR REDUCTION OF METHANE EMISSIONS FROM THE RUMEN

Since methane is a final product of rumen fermentation, strategies for reducing methane emissions from the rumen involve altering patterns of rumen fermentation. Because  $H_2$  and formate are principally utilized for methanogenesis in the rumen, most strategies primarily target the reduction of  $H_2$  and formate in the rumen. However, factors that alter rumen fermentation by the rumen microbial ecosystem are complicated. Any method to suppress methane production in the rumen must be accompanied by a method to convert the resulting accumulated  $H_2$  into other fermentation products. Otherwise, accumulated  $H_2$ , a waste product of rumen fermentation, would suppress rumen digestion (Wolin et al., 1997). Moreover, attempts for reducing methane emissions should be carefully evaluated in order to prevent disorders of rumen fermentation (Russell and Rychlik, 2001). Fundamentally, modification of rumen fermentation could influence methane production (Nagaraja et al., 1997; Weimer, 1998). Here we attempt to describe factors that could be used to reduce methane from the rumen.

#### Types of feed

*Estimation of methane production from components included in diets*: It is well known that methane production is influenced by the quality and quantity of feedstuffs. In this regard, methane production is often expressed by equations derived from experimental observations. Blaxter and Clapperton (1965) demonstrated that methane production in sheep and cattle is related both to dietary energy digestibility and feeding levels. They showed this relationship by the equation:

$$\begin{aligned} CH_4 \text{ (kcal/100 kcal GE)} \\ = 1.30+0.112D+L (2.37-0.050D) \end{aligned} \quad (2)$$

Where GE represents gross energy; D, digestibility; and L, levels of energy intake ("L = 1" means the minimal

maintenance level). According to equation 2, when L = 3, high dietary energy digestibility leads to low methane production.

Shibata et al. (1992) demonstrated that methane production could be accounted for in terms of dry matter intake only. They predicted methane production by the equation (Shibata et al., 1993):

$$CH_4 \text{ (L/day)} = -17.766+42.793DMI-0.849DMI^2 \quad (3)$$

where DMI represents dry matter intake (kg/day). Moreover, Kurihara et al. (2002) proposed that the methane conversion rate (MCR; MJ  $CH_4$  energy per 100 MJ gross energy intake) could be predicted by the following equations:

$$MCR = 3.37-0.272CP+0.119DMD \quad (4)$$

when the energy intake level of cows is less than 1.5 M;

$$MCR = 6.34-0.427CP+0.095DMD \quad (5)$$

when the energy intake level of cows is between 1.5 M and 2.5 M; and

$$MCR = 13.81-0.668CP-0.195NFE+0.203DMD \quad (6)$$

When the energy intake level of cows is greater than 2.5 M, where M represents the maintenance level of energy intake; CP, crude protein; DMD, dry matter digestibility (%); and NFE, nitrogen free extracts.

Equations 4-6 show that CP has a negative effect on methane production.

*Forage-based diets*: Ruminants fed with forage-based diets, the majority of the world's ruminants, face problems associated with low-quality diets. Leng (1993) summarized some approaches to solving the problems in view of methane reduction from the rumen, emphasizing that the production of ruminants fed poor-quality forages is limited by a low protein supply from the microbial ecosystem and a virtual absence of dietary bypass protein. In methane production from ruminants fed poor-quality forages, methane output relative to product output of ruminants depends on two factors:

- The efficiency of fermentative digestion in the rumen.
- The efficiency of conversion of feed to product (e.g. milk, beef), which in turn depends on the balance of nutrients absorbed (Leng, 1993).

To improve the rumen digestibility of forage, various modifications by physical processing such as grinding and pelleting, and chemical processing by chemicals such as ammonia and sodium hydroxide have been shown effective (Fahey et al., 1993). Whereas  $CH_4$  production is not

influenced as much by the type of carbohydrate at low intake as at higher intake (Kurihara et al., 1997), the type of forage in high-forage-intake diets can influence methane emission. For example, feeding high-quality alfalfa and corn silage can decrease methane production by 10 to 20% in a large-scale dairy farming system (Kume, 2002). Seasonal variations of pasture in methane production have been reported (Ulyatt et al., 2002; Lovett et al., 2006); hence, seasonal changes in the quality of pasture may affect methane emissions from grazing ruminants. It would be expected that altering forage quality could decrease methane production in the range of 20-40% (Leng, 1993; Hegarty, 2002).

**Grain-based diets** : Cereal grains, which contain much more storage carbohydrates than forage, are easily digested by rumen microorganisms such as starch-fermenting bacteria. Higher proportions of concentrates in the feed decrease methane emissions (Yan et al., 2000). This reduction in methane concentrate intake can be explained in part by equation 2, because high-energy contents in concentrates reduce dry matter intake.

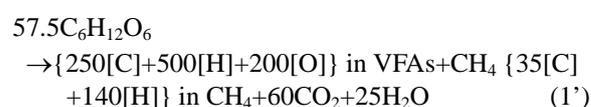
Starch in cereal grains is readily fermented by rumen microorganisms. It is well known that, as a consequence of starch fermentation, both the acetate to propionate (A/P) ratio and the pH in the rumen are decreased (Russell, 1998). While some starch-digesting bacteria produce significant amounts of propionate, many fiber-digesting bacteria produce large amounts of succinate, which is finally converted to propionate (Hungate, 1966). Hungate also reported that when large amounts of concentrates are present in the diet, 23% of propionate is produced from pyruvate via acryl CoA (the acrylate pathway) (Figure 1), whereas, in a forage-based diet, 92% of propionate is produced from pyruvate via succinate, (Satter et al., 1964). Therefore, it can be assumed that some starch-digesting bacteria produce lactate, which is eventually converted to propionate by lactate-utilizing bacteria such as *Megasphaera elsdenii*, which can produce propionate using the acrylate pathway (Counotte et al., 1981). Indeed, *Streptococcus bovis*, one of major starch-digesting bacteria in the rumen, release a great deal of lactate into the rumen (Asanuma and Hino, 2000). Russell suggested that rumen bacteria that produce propionate are more sensitive to pH than some bacteria that produce acetate and H<sub>2</sub>, because the A/P ratio was dramatically increased and a large amount of H<sub>2</sub> was detected when the final pH in his experiments was less than 5.3. Moreover, over the final pH range of 6.5 to 5.3, CH<sub>4</sub> production was highly correlated with A/P ratio, which depended on the pH and substrate (CH<sub>4</sub> = 0.02+0.05 pH; r<sup>2</sup> = 0.08) (Russell, 1998). Therefore, lactate accumulation in the rumen (depression of pH) would be caused by the slow conversion rate of lactate-utilizing bacteria, which change lactate to propionate and are

negatively sensitive to low pH. It has been reported that the numbers of lactate-producing and lactate-utilizing bacteria in the rumen change during adaptation from a low- to a high-concentrate diet (Mackie and Gilchrist, 1979; Tajima et al., 2001). Moreover, it is known that low pH in the rumen has selective actions on rumen microorganisms, especially on cellulolytic bacteria (Stewart, 1977; Russell and Dombrowski, 1980; Slyter, 1986).

As mentioned above, rumen fermentation is typically expressed by equation 1 (Wolin, 1979):



This equation can be converted into:



Therefore, the [H] in hexose would be divided into methane (20.3% [H]) and VFAs (72.5% [H]), if rumen fermentation proceeds according to equation 1. When hydrogen-producing reactions (acetate production) decrease and hydrogen-consuming reactions (propionate production) increase (Figure 1), the A/P ratio decreases, and methane production decreases because less hydrogen is available for methane production.

### Compounds (Feed additives)

Various compounds have been added to feed as additives specifically to abate methane emission from the rumen. These compounds can be categorized by their mode of action on methane production.

**Directly toxic to methanogens** : Halogenated methane analogues (e.g. bromochloromethane (BCM)) can inhibit methanogenesis by reacting with coenzyme B (cobamine), which functions at the last step of the methanogenic pathway (Chalupa, 1977; McCrabb et al., 1997; Shima et al., 2002). Although BCM is too volatile to be used as a feed additive, BCM- $\alpha$ -cyclodextrin complex could extend the period of effectiveness of BCM in the rumen (McCrabb et al., 1997). 2-bromoethanesulphonate (BES), a structural analogue of coenzyme M, and 3-bromopropanesulphonate (BPS), a potent inhibitor of methyl-CoM reductase, are known as chemicals that could inhibit methane production in the rumen (Ungerfeld et al., 2004). Because BCM, a kind of halogenated compound, is listed in the regulations of the "Montreal Protocol on Substances that Deplete the Ozone Layer (1987)" by the United Nations Environment Programme (<http://hq.unep.org/ozone/Montreal-Protocol/Montreal-Protocol2000.shtml>), application of these compounds should be carefully regulated.

Mevastatin and lavastatin, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, can inhibit growth and methane production of *Methanobrevibacter* strains isolated from the rumen (Miller and Wolin, 2001). Since *Archaea* are the only bacteria known to possess biosynthetic HMG-CoA reductase, HMG-CoA reductase inhibitors would have the potential to specifically inhibit rumen methanogens without inhibiting other rumen bacteria (Miller and Wolin, 2001).

Inhibitors directly toxic to methanogens are a powerful tool to stop methanogens from producing methane. However, as a result of these inhibitors, H<sub>2</sub>, which could suppress the activity of rumen fermentation (Wolin et al., 1997), can be expected to accumulate in the rumen.

*Antimicrobial reagents* : It has been believed that antimicrobial reagents such as antibiotics and bacteriocins are able to modify rumen fermentation. Antibiotics including ionophores and non-ionophores have been intensively studied for improvement of feed efficiency and animal health (Nagaraja et al., 1997).

Ionophores such as monensin and lasalocid have been known as one of most effective rumen modifiers; not only do they abate methane emission but also alter the various aspects of rumen fermentation. Ionophores are generally effective against Gram-positive bacteria but exhibit little or no activity against Gram-negative bacteria and methanogens in the rumen (Nagaraja et al., 1997). Furthermore, entodiniomorphs (*Entodinium*, *Diplodinium* and *Ophryoscolex*) in rumen ciliates are sensitive to ionophores (Dennis et al., 1986). It is assumed that ionophores are able to modify rumen fermentation based on their antimicrobial spectrum, which has been examined using culturable strains. Although a considerable number of rumen bacteria are unculturable, the antimicrobial spectrum can be deduced from the fact that hydrogen and formate producers (*Lachnospira multiparus*, *Ruminococcus albus* and *Ruminococcus flavefaciens*), butyrate producers (*Butyrivibrio fibrisolvens*, *Eubacterium cellulosolvens* and *Eubacterium ruminantium*), lactate producers (*Lactobacillus ruminis*, *Lactobacillus vitulinus* and *Streptococcus bovis*) and ammonia producers (*Clostridium aminophilum*, *Clostridium sticklandii* and *Peptostreptococcus anaerobius*) are susceptible to ionophores because of their Gram-positive cell walls, but succinate and propionate producers (*Anaerovibrio lipolytica*, *Fibrobacter succinogenes*, *Megasphaera elsdenii*, *Prevotella ruminicola*, *Selenomonas ruminantium*, *Succinimonas amylolytica* and *Succinivibrio dextrinosolvens*) are resistant to ionophores (Nagaraja et al., 1997). Therefore, the antimicrobial spectrum of rumen bacteria to ionophores would lead to the following changes in rumen fermentation:

- Enhancement of propionate production and reduction

of methane production.

- Improvement of nitrogen metabolism in the rumen.
- Reduction of lactate production.

On the other hand, it has been known that prolonged application of monensin to steers lost its methane-suppressing activity (McCaughey et al., 1997) and rumen bacteria developed readily resistance to ionophores (Newbold et al., 1993). Because many researchers have been strongly interested in ionophores, a number of review papers have been published (Nagaraja et al., 1997; Russell and Houlihan, 2003; Tedeschi, 2003). One peptide ionophore, aibellin, was able to increase propionate production in the rumen without significantly affecting production of total VFA, protozoal survival, or cellulose digestion (Hino et al., 1993; Hino et al., 1994).

Apart from antibiotics, bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5, has shown a capacity to reduce ruminal methane production *in vitro* (Lee et al., 2002).

*Balance of hydrogen-producing and hydrogen-consuming reactions in the rumen* : As shown in Figure 1, hydrogen is produced in several steps of rumen fermentation and flows into methane production, propionate production or butyrate production. It is known that many cellulolytic bacteria, protozoa and fungi in the rumen produce hydrogen in the process of carbohydrate degradation (Orpin and Joblin, 1997; Stewart et al., 1997; Williams and Coleman, 1997). Although it could be possible that repression of hydrogen-producing reactions leads to abatement of methane production, repression of hydrogen-producing reactions means repression of the activity of rumen fermentation and leads to restrained digestibility of carbohydrates and suppression of microbial growth. Thus, in order to reduce the flow of hydrogen into methane production, the flow of hydrogen should be diverted into propionate production via lactate or fumarate (Asanuma et al., 1999).

To alter the flow of hydrogen in the rumen, propionate precursors ("propionate enhancers"), which are intermediates of the fermentation pathways that lead to propionate, have been investigated. It has been reported that fumarate (Asanuma et al., 1999; Carro and Ranilla, 2003; Garcia-Martinez et al., 2005; Newbold et al., 2005) and malate (Carro and Ranilla, 2003; Gomez et al., 2005) could be useful for methane reduction in the rumen. *Veillonella parvula*, *Selenomonas ruminantium* subsp. *ruminantium*, *Selenomonas ruminantium* subsp. *lactilytica* and *Fibrobacter succinogenes* have shown a high capacity to reduce fumarate and should support propionate production in the rumen as fumarate-reducing bacteria (Asanuma and Hino, 2000). Additionally, intermediates in the conversion of pyruvate into butyrate ("butyrate enhancers") also could consume hydrogen as electron sinks (Ungerfeld et al., 2003).

Reduction of nitrate in the rumen is one of the important pathways yielding ammonia, which is utilized by rumen bacteria as a nitrogen source. The nitrate reduction (nitrite production) and nitrite reduction (ammonia production) pathways consume 4[2H] for reduction of one mole nitrate. The rate of nitrate reduction (nitrite production) is generally faster than that of nitrite reduction (ammonia production) (Iwamoto et al., 1999), but nitrite may accumulate in the rumen when levels of nitrate in the diet are high. Accumulation of nitrite in the rumen may cause decrease of oxygen transport in the blood (Dawson et al., 1997). Therefore, application of nitrate for methane reduction must be carefully controlled to avoid the toxicity of nitrite (Iwamoto et al., 1999; Yoshii et al., 2005).

**Other methods :** The suppressive effects of oils rich in medium-chain fatty acids (MCFAs) on methane production has been observed (Dong et al., 1997; Dohme et al., 2000). It was assumed that oils such as coconut oil suppress methanogens and/or ciliate (Dong et al., 1997; Dohme et al., 2000; Dohme et al., 2001). Comparing the effects of various MCFAs (8:0, 10:0, 12:0, 14:0) *in vitro*, 12:0 and 14:0 were identified to be most effective against rumen methanogens and methanogenesis (Dohme et al., 2001). Machmüller et al. (2003) reported that the methane-suppressing effect of coconut oil seems to be mediated through a changed metabolic activity and/or composition of the rumen methanogenic population.

Yeast cultures based on *Saccharomyces cerevisiae* are known as modifiers of rumen fermentation (Nagaraja et al., 1997) and may reduce methane production (Martin et al., 1989; Lila et al., 2004). However, in many cases, it has been difficult to statistically prove the effects of yeast cultures on rumen fermentation (Nagaraja et al., 1997). Oxygen consumption by respiring yeast in the rumen appears to be at least partly responsible for the probiotic activity of yeast cultures (Newbold et al., 1996).

Many compounds extracted from plants have been screened and utilized for altering rumen fermentation, because of increasing awareness of the hazards associated the use of antibiotics and chemical feed additives (Wallace, 2004) and because of efforts to promote more effective use of byproducts from regional industries. Some such products have shown utility for abatement of methane emission from the rumen. For example, it has been reported that saponins (Lila et al., 2003; Wallace, 2004; Hu et al., 2005), garlic oil (Busquet et al., 2005), Japanese horseradish oil (Mohammed et al., 2004), "Rumen-up" (Wallace, 2004), and others (Busquet et al., 2005; Busquet et al., 2006; Newbold and Rode, 2006) reduce methane emissions.

#### Other strategies

**Elimination of rumen ciliate :** Since part of the methanogens in the rumen cohabit with ciliate protozoa (see

above) and have been shown to be responsible for between 9-25% of methanogenesis in rumen fluid (Newbold et al., 1995), elimination of ciliate from the rumen can reduce methane emission from the rumen (Whitelaw et al., 1984; Ushida and Jouany, 1996). Although elimination of ciliate from the rumen can be performed by using chemicals, the possibility of biological control for ciliate populations in the rumen has been proposed by Klieve and Hegarty (1999).

**Immunization :** Vaccines against *Streptococcus bovis* and *Lactobacillus* spp in the rumen were attempted to prevent lactic acidosis in ruminants and could lead to a specific immune response demonstrated by the presence of specific antibody in the rumen fluid (Gill et al., 2000; Shu et al., 2000). Likewise, vaccines using some strains of rumen methanogens as antigens have been applied to methane abatement (Wright et al., 2004). In one experiment, one of two different vaccine formulations showed a significant (7.7%) reduction in methane emissions of sheep (Wright et al., 2004).

**Others :** Bacteriophages against rumen bacteria, not rumen archaea, have been detected in the rumen (Hoogenraad et al., 1967; Swain et al., 1996; Klieve et al., 2004); the presence of phages against archaea in the rumen has also been suggested (Newbold et al., 1996).

While attempts to oxidize methane in the rumen seem promising, such efforts have achieved oxidation of only 0.2-0.5% of the methane produced there (Kajikawa et al., 2003). It was assumed in that study that methane could be oxidized anaerobically in the rumen by reverse methanogenesis in consort with sulphate reduction (Kajikawa et al., 2003). Moreover, because PCR using methanotroph-specific primers could not detect methanotrophic bacteria capable of the aerobic oxidation of methane from the surface of the rumen wall, through which oxygen is supplied (Mitsumori et al., 2002), aerobic oxidation of methane may not occur in the rumen.

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