Mitigation of Methane Emission and Energy Recycling in Animal Agricultural Systems*

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ABSTRACT : Abatement of greenhouse gas emitted from ruminants and promotion of biogas energy from animal effluent were comprehensively examined in each anaerobic fermentation reactor and animal experiments. Moreover, the energy conversion efficiency of biomass energy to power generation were evaluated with a gas engine generator or proton exchange membrane fuel cell (PEMFC). To mitigate safely rumen methanogenesis with nutritional manipulation the suppressing effects of some strains of lactic acid bacteria and yeast, bacteriocin, β1-4 galactooligosaccharide, plant extracts (*Yucca schidigera* and *Quillaja saponarea*), L-cysteine and/or nitrate on rumen methane emission were compared with antibiotics. For *in vitro* trials, cumulative methane production was evaluated using the continuous fermented gas qualification system inoculated with the strained rumen fluid from rumen fistulated Holstein cows. For *in vivo*, four sequential ventilated head cages equipped with a fully automated gas analyzing system were used to examine the manipulating effects of β1-4 galactooligosaccharide, lactic acid bacteria (*Leuconostoc mesenteroides subsp. mesenteroides*), yeast (*Trichosporon serticeum*), nisin and *Yucca schidigera* and/or nitrate on rumen methanogenesis. Furthermore, biogas energy recycled from animal effluent was evaluated with anaerobic bioreactors. Utilization of recycled energy as fuel for a co-generator and fuel cell was tested in the thermophilic biogas plant system. From the results of *in vitro* and *in vivo* trials, nitrate was shown to be a strong methane suppressor, although nitrate *per se* is hazardous. L-cysteine could remove this risk. β1-4 galactooligosaccharide, *Candida kefyr*, nisin, *Yucca schidigera* and *Quillaja saponarea* are thought to possibly control methanogenesis in the rumen. It is possible to simulate the available energy recycled through animal effluent from feed energy resources by making total energy balance sheets of the process from feed energy to recycled energy. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1199-1208)***

Key Words : Methane, *Yucca schidigera*, *Quillaja saponarea*, Oligosaccharide, Fuel Cell, Biogas Plant

INTRODUCTION

Global warming, due to an increase in the atmospheric concentration of greenhouse gases, is an important issue (Takahashi and Young, 2002). The worldwide trends of carbon dioxide have shown an increase in the greenhouse effect on global warming (Houghton, 1994). However, methane is an important greenhouse gas, second only to carbon dioxide in its contribution to global warming, due to its high absorption ability of infrared in radiation from the sun (IPCC, 1994). The world population of ruminants is an important source of methane, contributing approximately 15% of the total atmospheric methane flux. The control of methane emission is a logical option since atmospheric methane concentration is increasing at a faster rate than carbon dioxide (Moss, 1993).

Methanogens in the rumen are hydrogenotrophic bacteria which carbon dioxide is chiefly reduced with hydrogen though the acetotrophic methanogens in the biogas system generate the methane from acetic acid, formic acid, methanol, and the methylamine, etc. The reduction is a significant electron sink in the rumen ecosystem (Klieve and Hegarty, 1999). The mechanism is a complex enzyme reaction consisting of seven stages (DiMarco et al., 1990). Methane contains 892.6 kJ combustible energy per molecule at 25°C and 1,013 hPa, while not contributing to the total supply of metabolic energy to ruminants (Takahashi et al., 1997). As reported by Leng (1991), methane production from ruminants in the developing countries may be high since the diets are often deficient in critical nutrients for efficient microbial growth in the rumen. A number of inhibitors of methanogenesis have been developed to improve feed conversion efficiency of ruminant feeds claimed to be effective in suppressing methanogens or overall bacterial activities (Chalupa, 1984). Attempts to reduce methanogenesis by the supplementation of chemicals such as ionophores (monensin and lasalocid), have long been made (Hopgood and Walker, 1967; Chalupa, 1984). However, these ionophores may depress fiber digestion and protozoal growths (Chen and Wolin, 1979). In

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addition, some resistant bacteria will appear in the rumen from the results of long term use of the ionophores.

The fiber bacteria that use structural carbohydrates such as cellulose and hemicellulose produce this hydrogen. When the hydrogen accumulates in large quantities in the rumen, the proliferation and activities of rumen microorganisms are inhibited by high pressure of hydrogen. The generation of methane by methanogens has an important implication on the removal of harmful hydrogen for the rumen microorganism (Interspecies Hydrogen Transfer) (Miller, 1995; Hegarty and Gerdes, 1999). However, rumen methanogenesis becomes a big load in environmental preservation. Therefore, it is a global issue to control rumen methanogenesis, which is not only for animal agriculture but also, with respect to the prevention of global warming (Takahashi, 2001; Takahashi et al., 2001).

On the other hand, methane included in the biogas generated from anaerobic fermentation of animal effluent is convertible to an alternative energy source of fossil fuel. Furthermore, hydrogen reformed from methane can be used for fuel cell power generation as fuel supply. Whilst rumen methanogenesis must be, therefore, reduced to abate greenhouse effect, the potential energy of effluent should be recycled as a useful alternative energy source to reduce fossil energy consumption.

The present paper deals with some nutritional options using some probiotics and natural compounds compared with antibiotics to abate methane emission from farm animals, especially ruminants and the recycling rate of the potential energy of effluent as a fuel source of a co-generator and fuel cell.

RUMEN METHANE MITIGATION

Alternative reductions for the abatement of methanogenesis in the rumen

Theoretically, methanogenesis can be reduced by either a decrease in the production of H_2, the major substrates for methane formation or an increase in the utilization of H_2 and formate by organisms other than methanogens. However, direct inhibition of H_2-forming reactions may depress fermentation in microorganisms that produce H_2, including main cellulolytic bacteria such as *Ruminococcus albus* and *Ruminococcus flavefaciens* (Wolin, 1975; Belaich et al., 1990). Therefore, a reduction in H_2 production by the enhancement of reactions that accept electrons is desirable (Stewart and Bryant, 1988). Jones (1972), Allison et al. (1981) and Takahashi et al. (1983) showed that nitrate as a hydrogen (electron) acceptor competed with reducing steps in methane production and, consequently, markedly suppress methanogenesis by rumen microbes.

However, elevated levels of nitrate in forages could pose a serious threat to animal due to its conversion to toxin nitrite. Ruminants are particularly vulnerable to nitrate intoxication as nitrate is primarily reduced to nitrite by NADPH-nitrate reductase (*EC* 1.6.6.3) of nitrate reducing bacteria in rumen, and then to ammonia via hydroxylamine. Subsequently, nitrite accumulated in the rumen is absorbed in the blood stream to produce methaemoglobin as a result of the way of oxidative properties of nitrite against ferrous forms of oxyhemoglobin (Allison and Reddy, 1984). With an advance of methaemoglobinemia, oxygen consumption of ruminants decreases because of disruption to oxygen transportation (Takahashi et al., 1983) and pulmonary of gaseous exchange and metabolic rate are altered, whereby indicating the extent of the physiological effects of nitrite on the animal as a whole (Takahashi and Young, 1991). Additionally, the reduction of nitrate in the rumen may alter the oxidation-reduction (redox) potential and the molecular proportion of volatile fatty acid (VFA) (Jamieson, 1958; Allison et al., 1981; Takahashi et al., 1983, 1989).

An approach to the solution of this problem may be the depression of the nitrite formation rate by means of an inhibition of the enzymatic activity of nitrate reductase in the rumen microbes, requiring molybdenum (Metzler, 1977) to catalyze the primary step to nitrite in the assimilatory reduction of nitrate. To this effect, in the studies using tungsten (W), it was clarified that the interfering incorporation of molybdenum into the bacterial nitrate reductase was the most efficient to inhibit nitrate reduction in the rumen (Prins et al., 1980; Korzeniowski et al., 1981; Marais et al., 1988; Takahashi et al., 1989). Tungsten may, however, have a slight application in animal feeding as a prophylactic against nitrate poisoning owing to its potential toxic properties (Takahashi and Young, 1991, 1992). Additionally, it has been reported in *in vitro* and *in vivo* experiments that the adverse effect of nitrate on ruminant physiology could be counteracted by L-cysteine. L-cysteine is degraded in rumen by a microbial cystathionine γ-lyase to generate sulphide, then, sulphur ion generated from sulphide can bind to W to form an insoluble inorganic compound. Consequently, the activity of nitrate reductase us inhibited (Takahashi, 1989; Takahashi et al., 1989; Takahashi and Young, 1991). Also, as reported by Takahashi et al. (1997), methane emission in sheep was suppressed by 13% by L-cysteine. Thus, methanogenesis is possibly suppressed by the combination between nitrate and L-cysteine without toxicity of nitrate.

Oligosaccharides are naturally occurring carbohydrates with a low degree of polymerisation and consequently low molecular weight, being commonly found to perform in the various plant and animal sources. β1-4 galactooligosaccharides (GOS) are non-digestible carbohydrates, which are resistant to gastrointestinal digestive enzymes, but fermented by specific colonic bacteria. The products of fermentation of GOS in the colon, mainly short chain fatty acids, have a

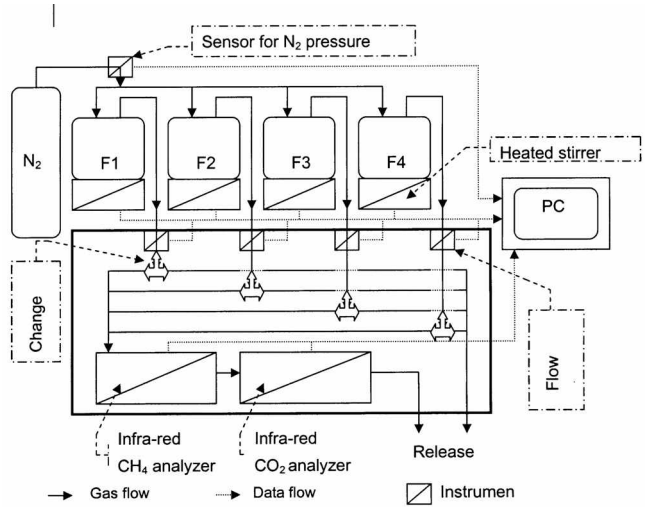

Figure 1. The continuous fermented gas qualification system.

role in the improvement of the colonic environment, energy supply to the colonic epithelium, and calcium and magnesium absorption (Sako et al., 1999). The indigestibility and stability of GOS to hydrolysis by α-amylase of human saliva, pig pancreas, rat small intestinal contents and human artificial gastric juice has been shown in several *in vitro* experiments (Ohtsuka et al., 1990; Watanuki et al., 1996). This is because GOS have β-configuration, whereas human gastrointestinal digestive enzymes are mostly specific for α-glycosidic bonds. From this point of view, expectedly, GOS could be readily degraded in the rumen as a result of the ruminal enzymes being specific for β-glycosidic bonds (Sar et al., 2004). Thus, lactic acid bacteria may consume GOS to promote propionate formation through acrylate pathway, and consequently the competition with both methanogens and process of nitrate reduction for hydrogen will occur.

Previous studies have suggested that yeast culture supplements can exert significant effects on the performance of ruminants. *Saccharomyces cerevisiae* culture has been used as dietary supplement in ruminant production for many years. However, interest in *S. cerevisiae* culture as a potential alternative to antimicrobial feed additives has increased within the last decade (Van Navel and Demyer, 1988; Sullivan and Martin, 1999). Some of beneficial effects include an increase in feed intake (Ruf et al., 1953; Phillips and VonTungeln, 1985), milk production (Hoyos et al., 1987; Teh et al., 1987) and weight gain (Greive, 1979; Fallon and Harte, 1987). Yeast cultures may alter the patterns of VFA formation (Teh et al., 1987; Wiedmeier et al., 1987; Martin et al., 1989) and ruminal pH (Harrison et al., 1988), increase the concentrations of anaerobic and cellulolytic bacteria (Weidmeier et al., 1987; Harrison et al., 1988) and alter digestion (Gomez-Alarcon *et al.*, 1987). In addition, yeast culture (Diamond V Yeast culture, Diamond V Mills, Inc, Cedar Rapids, IA 52407) has received considerable attention as nitrate intoxication preventive among livestock producers in Oklahoma (Stone, 1974). Because yeast culture is very palatable, it could readily be utilized in many management system (Streeter et al., 1981). As reported by Gerald and Keith (1984), however, the yeast culture was indicated not to decrease the methaemoglobinemia produced by consumption of high-nitrate forages by sheep or cattle. As the compositions of the cultured yeast used in many of these studies were quite variable, consequently, response of animal performance was quite variable. These fluctuations of the experimental results mainly attributed to wide varieties of strain of yeast culture and diets used *in vivo* trials. Therefore, the screening of manipulators in *in vitro* system should be established under the same incubation conditions.

Effects of β1-4 galacto-oligosaccharide, L-cysteine and yeast cultures and lactic acid bacteria on rumen methanogenesis and nitrate reduction *in vitro*

In vitro continuous fermented gas qualification system (Takahashi et al., 2003) was originally designed to run these experiments based on the early *in vitro* model for Gas Production System (Takahashi et al., 2000). Figure 1 shows the schematic explanations.

Rumen fluid harvested from two rumen fistulated cows was pooled and strained through woven nylon cloth. Then, 400 ml of strained rumen fluid was mixed with 400 ml of autoclaved buffered artificial saliva (McDougall, 1948). The buffer fluid was kept in the fermenters. The incubation was carried out anaerobically at 39°C for 12 h with the addition of 10 g of substrate consist of orchardgrass hay (DM 94.9%, CP 6.7%, GE 18.50 kJ g DM^{-1}), lucerne cube (DM 92.8%, CP 18.2%, GE 18.57 kJ g DM^{-1}) and concentrate mix. (DM 94.1%, CP 21.0%, GE 19.31 kJ g DM^{-1}) in the ratio at 2:1:1 on air dry matter basis with or without manipulator. β1-4 galactooligosaccharide (GOS, 200 mg L^{-1}) in powder form, $NaNO_3$ (NO3, 10 mM), and L-cysteine (CYS, 10 mM) were used as manipulators of rumen fermentation. To test the inhibitory effect of GOS, two different probiotics (two strains of yeast and lactic acid bacteria), and L-cysteine (CYS, 10 mM) on ruminal nitrate reduction, $NaNO_3$ (NO3, 10 mM) with or without GOS (200 mg L^{-1}), two types of cultured yeast and lactic acid were used in this experiment. Control incubations were conducted without any manipulators. The preparation of β1-4 galactooligosaccharide (Yakult Central Institute for Microbiological Research, Tokyo, Japan) contains 14% penta-and hexasaccharides, 24% tetrasaccharides, 40% trisaccharides, 10% disaccharides and 11% lactose.

Two strains of yeast (*Trichosporon sericeum*: Y7A and *Candida kefyr*: Y8A) and two strains of lactic acid bacteria (*Leuconostoc mesenteroides subsp. Mesenteriodes*: L5 and *Lactococcus lactis subsp. lactis*: L42) used as probiotics in

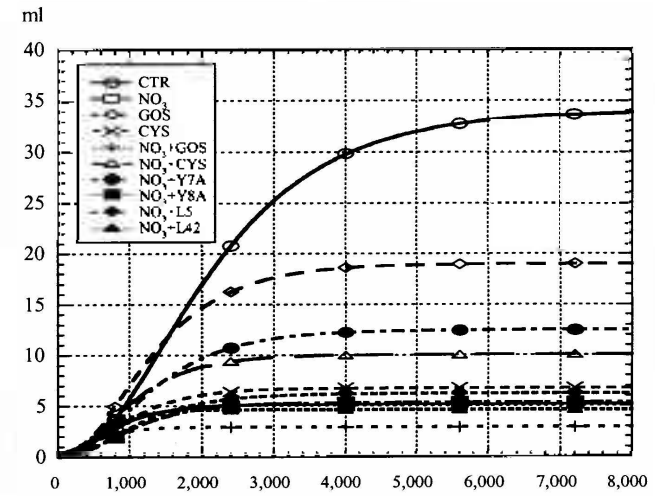

Figure 2. Effects of administration of nitrate, GOS, L-cysteine and probiotics on a cumulative methane production. CH_4 (ml) $a-b(1-e^{-ct})3$, t = min.

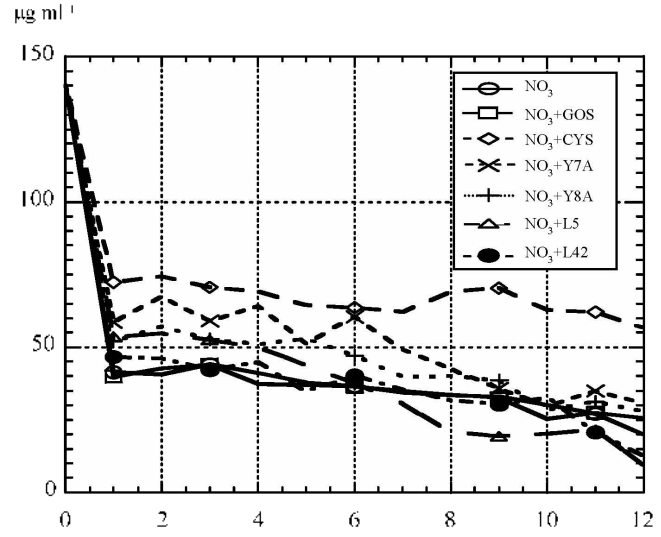

Figure 3. Effects of nitrate and/or GOS, L-cysteine and yeast cultures and lactic acid bacteria on nitrate (NO_3-N) reduction.

this trail were extracted from naturally-fermented milk "Laban" produced from sheep milk in Yemen. Y7A and Y8A, and L5 and L42 were prepared by growth on YM broth and MRS broth, respectively.

From the results of 12 hours incubation, cumulative methane production was extrapolated by non-linear regression model (Figure 2). Significantly ($p<0.05$) lower value of cumulative methane production was observed in all treatments compared to control incubation. The value in NO3 · GOS was the lowest among treatments. Moreover, the cumulative methane production was significantly ($p<0.05$) decreased when GOS and CYS were supplemented.

Figure 3 shows the time course of change of NO_3-N concentration in incubation fluid. After 12 h incubation, NO_3-N concentration in NO_3 · L5 tended to be lower, and

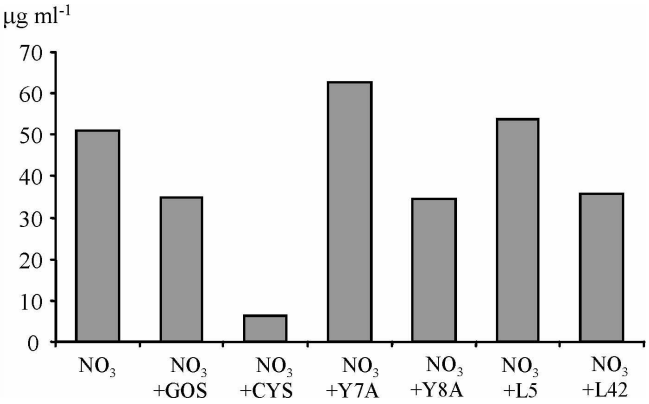

Figure 4. Effects of nitrate and/or GOS, L-cysteine, and yeast cultures and lactic acid bacteria on nitrite (NO_2-N) formation.

nitrate reduction rate in NO_3 · L5 treatment was promoted compared to NO_3 treatment.

Significantly ($p<0.05$) higher values of NO_3-N concentration were observed in NO_3–CYS treatment compared to NO_3 treatment. The maximum value of NO_2-N concentration in each treatment was estimated by non-linear regression of $Y = a-be^{(-(t-c)^2 d^2)}$ using practical values obtained from 12 hours incubation. Figure 4 shows that significantly ($p<0.05$) lower value of NO_2-N concentration in NO_3 · CYS treatment was observed compared to NO_3 treatment whereas GOS, Y8A, and L42 tended to decrease nitrite formation ($p>0.05$). Although the possible mechanism was not determined, the administration of nitrate with β1-4 galactooligosaccharide (GOS), yeast (*Candida kefyr* strain), lactic acid bacteria (*Lactococcus lactis subsp. lactis*) and L-cysteine were suggested to possibly control rumen methanogenesis and to prevent nitrite formation in rumen.

Effects of nitrate mixed with β1-4 galactooligosaccharide and Candida kefyr on methane emission in sheep

Four rumen-fistulated wethers (43.8-59.3 kg) were allocated to four dietary treatments in a 4×4 Latin square design and fed on a basal diet of chopped alfalfa hay cube and timothy hay (50:50, w/w) at a maintenance level (55 g DM $kg^{-0.75}$ body weight). All animals were individually maintained in metabolic crate equipped with a ventilated hood system to capture respiratory methane. To examine the effect of β1-4 galactooligosaccharide (GOS) and yeast (*Candida kefyr* strain: Y8A) culture on the nitrate-induced poisoning, nitrate with or without GOS and Y8A was directly administered into the rumen via fistula as single dose 30 min after the morning feeding. GOS and Y8A were supplemented by sprinkling onto the feed and through rumen fistula, respectively. Physiological saline (0.9% NaCl) was given as the control treatment. Respiratory gaseous exchanges were monitored from 1 h before to 7 h

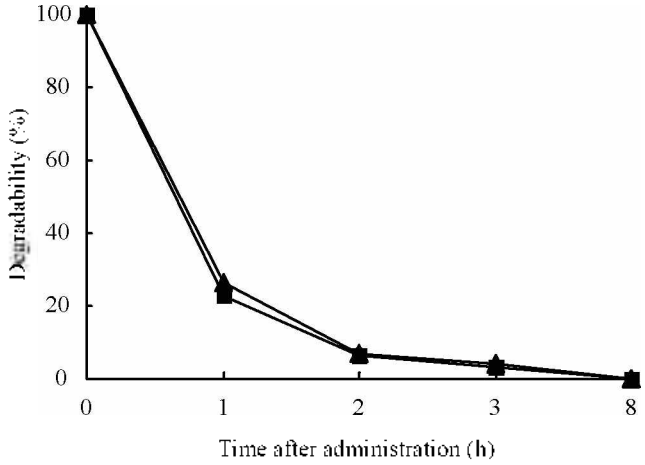

Figure 5. Rumen degradability of β1-4 galactooligosaccharide (GOS) in the presence of nitrate (■) and nitrate with *Candida kefyr* (▲).

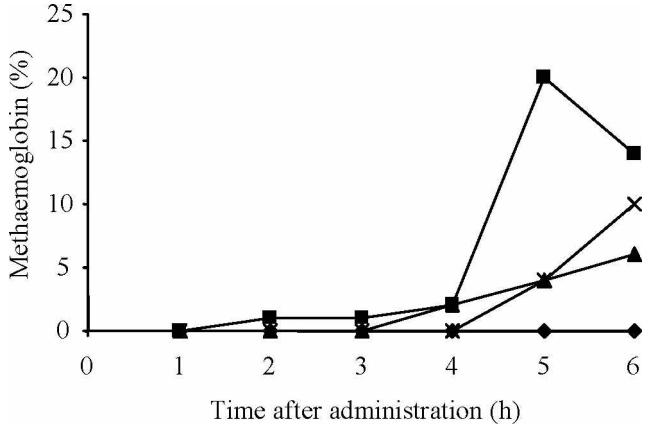

Figure 6. Time course of change of methaemoglobin formation (%) after administration of saline control (◆), nitrate (■), nitrate +GOS (▲) and nitrate+GOS+Y8A (×). Each point indicates a mean of four animals.

after administration of the chemicals. Venous blood samples were collected via a jugular catheter at 1, 2, 3, 4, 5 and 6 h and rumen fluid was withdrawn via rumen fistula 1, 2, 3, 4, 5, 6 and 7 h after administration of nitrate to check the development of nitrate-nitrite poisoning physiologically.

Figure 5 shows rumen degradability of GOS in the presence of nitrate and nitrate with *Candida kefyr*. Although GOS is resistant to gastrointestinal digestive enzymes, it is degradable in the rumen by more than 90% within 3 h.

Figure 6 shows the time course of change in methaemoglobin formation. The maximum formation of methaemoglobin in haemoglobin was observed 5 h after administration of nitrate to animal. For GOS-treated animals, methaemoglobin were prevented though significant difference was not observed ($p>0.05$). Methaemoglobin concentration for nitrate with GOS+Y8A was also lower than that for nitrate alone ($p>0.05$). No detectable concentration of methaemoglobin was formed in

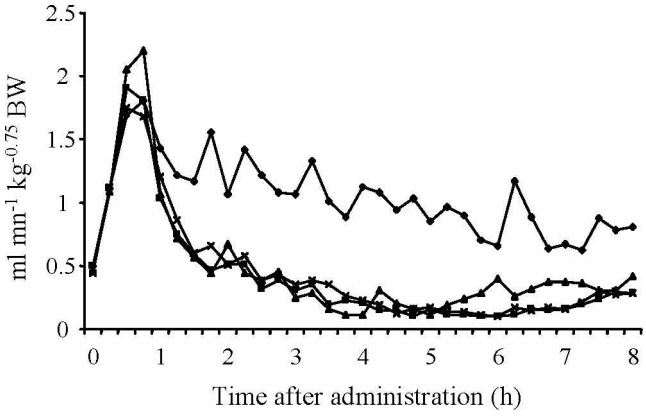

Figure 7. Time course of change of methane production after administration of saline control (◆), nitrate (■), nitrate+GOS (▲) and nitrate+GOS+Y8A (×). Each point indicates a mean of four animals.

the blood of the saline control.

Figure 7 shows the time course of change in methane production. Methane production decreased by 14% of the control value 5 h after administration of nitrate alone. Methane production for nitrate with GOS tended to be lower than that for nitrate alone 3-4 h and 5 h after administration. In addition, methane production for nitrate with GOS+Y8A treatment was lower than that for nitrate alone 2, 4.50 and 6.50 h after administration. However, for nitrate with GOS+Y8A treatment, methane production was not observed to lower than that for nitrate with GOS.

It has been suggested that for sheep treated with nitrate plus GOS, GOS was readily degraded and consumed by lactic acid bacteria promoting propionate formation through acrylate pathway. Hence, methane production was inhibited as a result of propionate production being in direct competition with methanogenesis for available hydrogen. However, methane production for the administration of nitrate plus GOS was not observed to be lower than that for nitrate treatment alone because of nitrate possibly suppressed methanogenesis at the maximum level. Additionally, rates of nitrate reduction and nitrite accumulation in sheep given with nitrate plus GOS were decreased, possibly, as a result of GOS competed with nitrate reduction for hydrogen to produce propionate through acrylate pathway. In consequence, methanogenesis by rumen microbes and methaemoglobin formation will be suppressed. On the contrary, rates of nitrate reduction and nitrite accumulation for sheep treated with nitrate plus GOS plus *Candida kefyr* were observed to be higher than that without *Candida kefyr*, although methanogenesis was similarly low. It has been suggested that nitrate-reducing bacteria such as *Selenomonas ruminantium*, *Veillonella parvula* and *Wollinela succinogenes* are likely to be enhanced by *Candida kefyr*.

Table 1. Effect of nisin on respiratory methane emission in sheep

CH_4	Control	Nisin
L kg $BW^{-0.75}$	1.79	1.60*
L kg DMI^{-1}	23.4	21.0*
L kg $DOMI^{-1}$	34.0	31.4*

* $p<0.01$ DMI: Dry matter intake.
DOMI: Digestible organic matter intake.

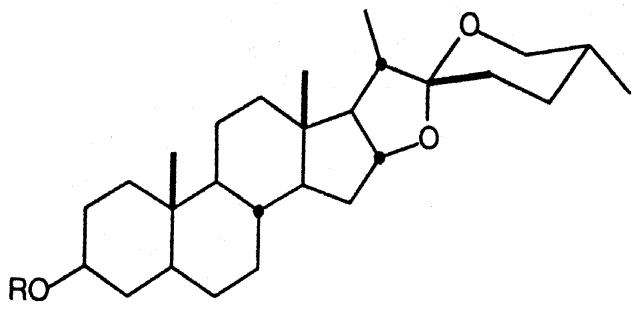

Figure 8. The structure of sarsaponin of *Yucca schidigera*.

OTHER NATURAL MANIPULATORS AS METHANE SUPPRESSORS

Bacteriocins being proteins and peptides synthesized on ribosome, exert bacteriostasis to affinity germ. Some strains of Lactobacillus produce bacteriocins. Nisin is a bacteriocine which is produced by *Lctococcus lactis*. link structured 34 amino acids are included in its structure. Its molecular weight is 3,510 Da. It is a hydrophobe peptide, and has a bacteriostasis to gram-positive bacteria. Especially, nisin effectively suppresses *Bacillus Clostridium* which is a deterioration germ caused deterioration of foods and poisoning. Moreover, nisin obstructs the germination of the bacterial spore.

Table 1 shows the results of respiratory methane emission in respiratory trials. Supplementing nisin decreased the respiratory methane emission significantly ($p<0.01$). *Yucca schidigera* which is the shrub plant grows naturally in the desert of southern part of United States and northern part of Mexican promote microbial protein synthesis and suppress methane in the rumen. Figure 8 shows that *Yucca schidigera*, used as a feed additive is rich in sarsaponin (steroid saponin). This kind of saponin adsorbs NH_3 when its concentration is high in the rumen and discharges NH_3 when it is low. This function improves nitrogen utilization efficiency in the ruminant animals.

In addition, sarsaponin has an affinity to water and lipids as a surface-active agent and it dissolves protozoa due to unavailable cell wall. Figure 9 shows chemical structure of triterpenoid saponin in *Quillaja saponarea*. As same as a *Yucca schidigera*, plenty of saponin compounds are included in the extract extracted from *Quillaja saponarea* which is a woody plant growing naturally in Chile. As for saponin in *Quillaja saponarea*, there are many kinds of the

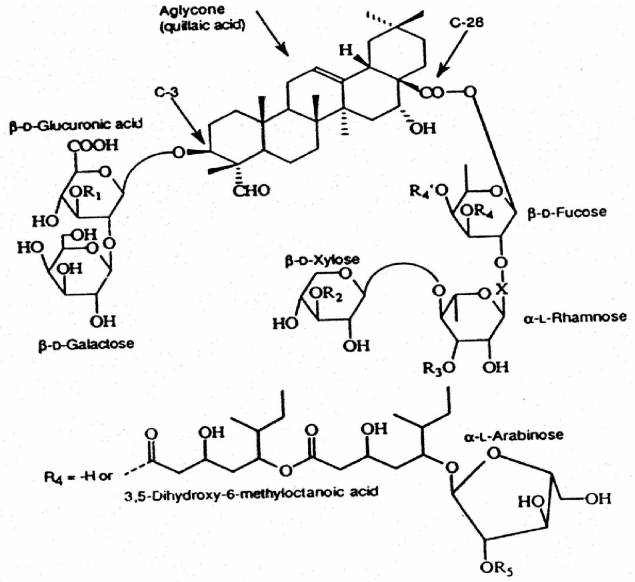

Figure 9. The structure of triterpenoid saponin in *Quillaja saponarea*.

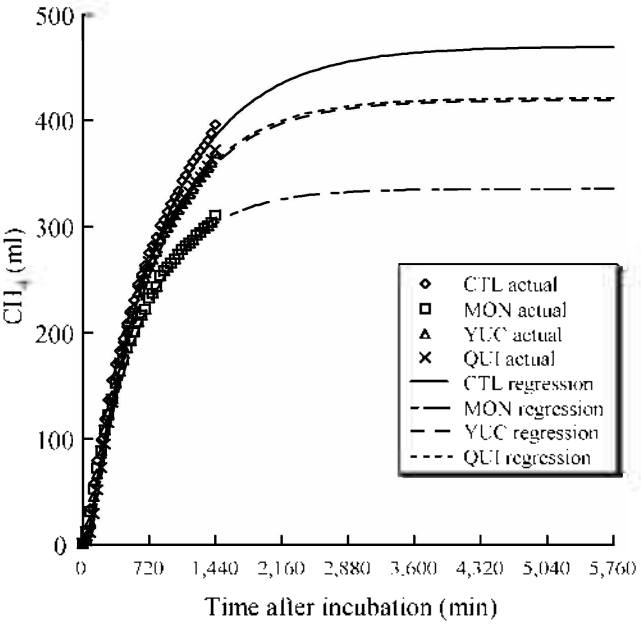

Figure 10. Effeects of Yucca scidigea, Quillaja saponarea and monensin on cumulative carbon dioxide *in vitro*.

saponin contained (50 kinds or more) compared with the *Yucca*, *Quillaja saponarea* is extremely excellent in interfacial revitalization.

Figure 10 shows cumulative methane production *in vitro*. As expected as a suppressor to methanogens monensin significantly ($p<0.05$) declined the cumulative methane production. *Yucca schidigera* and *Quillaja saponarea* decreased methanogenesis as well as monensin.

However, *Yucca schidigera* and *Quillaja saponarea* could stimulate the reducing bacteria other than methanogens, because they increased cumulative carbon

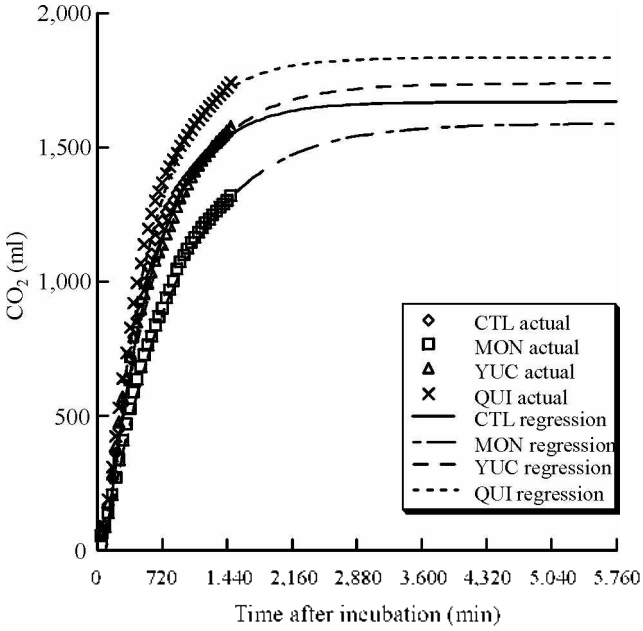

Figure 11. Effects of *Yucca schidigera*, *Quillaja saponarea* and monensin on cumulative production *in vitro*.

Table 2. Effect of *Yucca schidigera*, *Quillaja saponarea* and monensin on VFA and ammonia nitrogen production at 24 h incubation

	CTL	MON	YUC	QUI
Total VFA (mM)	43.03[a]	37.51[b]	46.14[a]	43.52[a]
Acetic	30.17[a]	24.95[b]	30.92[a]	30.25[a]
Propionic	7.67[c]	8.19[bc]	10.31[a]	8.62[b]
Iso-butyric	0.29	0.09	0.17	0.17
Butyric	3.63	3.11	3.53	3.23
Iso-valeric	0.41	0.36	0.39	0.38
Valeric	0.86	0.81	0.83	0.87
A/P ratio	3.93[a]	3.04[c]	3.01[c]	3.52[b]
Ammonia-N (mg L^{-1})	97.07[ab]	121.68[a]	74.33[b]	68.81[b]

[a, b, c] Means within the same row without common superscript differ significantly ($p<0.05$).

dioxide production as shown in Figure 11. Methanogens in the rumen are hydrogenotrophic bacteria, which produce methane by reducing carbon dioxide with hydrogen. Thus, hydrogen for methanogenesis was intercepted by other reduction reactions encouraged by *Yucca schidigera* and *Quillaja saponarea*, i.e. increases in total VFA and carbon dioxide production, and decrease in ammonia concentration, demonstrated that these saponin stimulated bacterial protein synthesis (Hussain and Cheeeke, 1995; Harinder et al., 1998) related to propionate production (Table 2). So far, *Yucca schidigera* has been reported to enhance ruminal propionate synthesis (Kil et al., 1994; Hristov et al., 1999). *Quillaja saponarea* was also demonstrated to increase propionate synthesis in the incubation trial. Steroid saponin and triterpenoid saponin in these plants could selectively emphasize rumen bacterial protein synthesis due to their ammonia absorbing abilities. Consequently, the cumulative

Figure 12. Biogas model plant in a farm attached to Obihiro University.

methane production was depressed by plant extracts. Since sarsaponin interferes with rumen cellulolytic bacteria to adhere feeds, acetate production is weakened by the saponin compound (Lila, 2003). However, both plant extracts did not affect the acetate production. For monensin, the decrease in methanogenesis was not only involved in enhancing propionate production, but also direct bacteriostasis to methanogens due to decrease in total VFA production and the cumulative carbon dioxide production.

In consequence, *Yucca schidigera* and *Quillaja saponarea* can be effective manipulators to safely mitigate rumen methanogenesis and urine nitrogen loss instead of ionophores.

RECYCLING OF METHANE AND NITROGEN FROM ANIMAL EFFLUENT

Anaerobic fermentation of animal effluent

To estimate the potential energy as methane recycled from animal manure excreted, the anaerobic fermentation trials were conducted using the thermophilic biogas reactors. In this experiment cow manure was used instead of sheep manure due to the difficulty of promoting anaerobic fermentation. Furthermore, the efficiency of electric power use of methane generated from the anaerobic fermentation of animal effluent was measured in the thermophilic biogas plant (Mitsui Engineering and Shipbuilding Co., Ltd.) with gas engine generator or proton exchange membrane fuel cell (PEMFC, Matsushita Electric Works, Ltd.).

Figure 12 shows the biogas model plant. This thermophilic biogas plant can produce 100 cubic meters biogas everyday from the slurry of 200 cows.

Figure 13 shows a portable PEMFC generator. The PEMFC generator consisted of desulfurizer, fuel processor, PEMFC stack, power supply unit, process controller and balance of plant devices (Adachi et al., 2002). The fuel

Figure 13. Biogas PEMFC Generator connected to the biogas model plant in Obihiro University.

processor integrated into the portable generator consistd of steam reformer, water gas shift reactor and preferential oxidizer and incorporated all three reactors into a compact body to adopt portable usage. In the present study the generator is connected to the biogas plant and being tested by using biogas that contains from 45% to 60% methane, from 40% to 55% carbon dioxide, small amount of oxygen and nitrogen and about 100 ppm of hydrogen sulfide. In order to apply to biogas, the fuel supply unit was modified and process control parameters in terms of mass flow and reactor temperatures were changed and optimized from original ones for butane. Also, capacity of the desulfurizer was increased because of about ten times higher sulfide concentration compared with a butane gas cylinder. All the other system is, however, basically the same with the original system.

Figure 14 shows biogas fuel processing scheme. Reformate composition achieves less than 10 ppm carbon monoxide and around 60% hydrogen along with carbon dioxide, methane and nitrogen. The Biogas PEMFC generator shows excellent performance compared with the original system for butane.

From the results of anaerobic fermentation trials of cow manure in the biogas reactors, 300 L pure methane equivalent to 11.85 MJ can be collected per each kg DM of manure. In the measurement of power generation efficiencies from cow effluent in the thermophilic biogas plant, the ratios in power generation, heat and loss were 28%, 33% and 39% in gas engine generator, and 38%, 40% and 22% in PEMFC, respectively. As the DM intake of 600 kg cow at maintenance level is ca 7.5 kg d^{-1}, the cow excreted 2.6 kg DM d^{-1} in her feces under 65 % digestibility. Thus, 31.1 MJ recycled energy can be withdrawn from the feces as methane. According to the power generation efficiencies, each electric power was estimated 8.7 MJ and 11.8 MJ in gas engine generator and PEMFC, *i.e.*, 100 W and 136 W may be recycled from 7.5 kg DM intake per day.

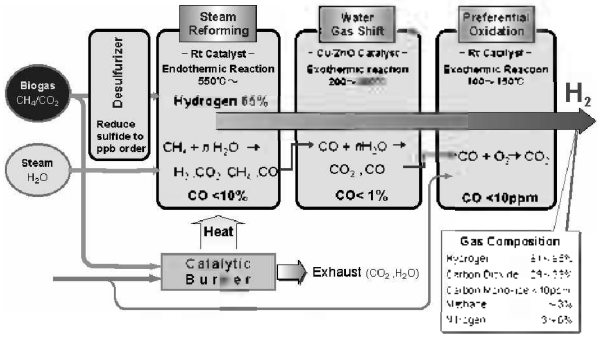

Figure 14. Fuel processing of biogas.

CONCLUSION

From the results of *in vitro* and *in vivo* trials, β1-4 galacto-oligosaccharide, *Candida kefyr*, nisin, *Yucca schidigera* and *Quillaja saponarea* were suggested to possibly control rumen methanogenesis in the rumen. β1-4 galactooligosaccharide and *Candida kefyr* as well as L-cysteine combined with nitrate may potentially suppress rumen methanogenesis and dysfunction attributed to nitrate. In the anaerobic fermentation with the biogas reactors 300 L pure methane equivalent to 11.85 MJ can be collected per each kg DM of manure. In the measurement of power generation efficiencies from cow effluent in the thermophilic biogas plant, the ratios in power generation, heat and loss were 28%, 33% and 39% in gas engine generator, and 38%, 40% and 22% in PEMFC, respectively. These recycled energy in power generation were simulated 9.8% in gas engine generator and 13.3% in PEMFC of gross energy consumed by cow.

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