Effect of Sodium Percarbonate on Methane Emission, Nutrient Digestibility, and Rumen Fermentation in Sheep

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ABSTRACT

The effects of sodium percarbonate on inhibiting methane emission were studied *in vitro* and *in vivo*. Four treatments were prepared as follows, 0.05mM, 0.1mM, and 0.5mM of H_2O_2 as final concentration in fermenting vessel and a control. These were incubated with rumen juice and buffer solution for 24 hours. Information on gas emission, characteristics of fermentation and protozoa population were observed. The result indicated that, sodium percarbonate could inhibit CH_4 production without negative impact on CO_2 production and production of volatile fatty acids. The use of 0.05mM of H_2O_2 was an optimum level for reducing methane emission without affecting other production parameters. Kleingrass and formula feed which were mixed of 70:30 as dry matter basis were fed for 4 sheep with 5g of sodium percarbonate as treatments (2.0mM as final concentration of H_2O_2 in the rumen) and without sodium percarbonate as control. Information on gas emission, digestibility, nitrogen balance, energy balance, characteristics of rumen fermentation and protozoa population were observed. Based on the result, sodium percarbonate could inhibit methane emission with sheep, but this amount of sodium percarbonate had negative impact on rumen microbes.

Key Words: Sodium percarbonate, Methane, Rrumen, Mitigation

INTRODUCTION

Methane is one of the major end products of fermentation in ruminants. Ruminants lose 2-12% of the gross energy they obtain from feed in the form of methane. Methane has about 25 times more global warming potential than carbon dioxide (CO_2). By improving nutritive value of feeds it is possible to reduce methane emission from ruminants and to mitigate global warming at the same time. The supernatant of Lactobacillus plantarum *TUA1490L* (LP) reduced *in vitro* CH_4 production, but the non-proteinaceous anti-microbial substance was not identified in our study (Asa et al., 2010), but has subsequently been shown to be hydrogen peroxide (H_2O_2).

The anti-microbial effect of H_2O_2 has been attributed to its strong oxidising effect on bacterial cells and to the destruction of the molecular structure of cell proteins Ito et al. (2003) and Zalán et al. (2005). However, there is no information available on the effect of H_2O_2 on rumen fermentation. Hydrogen peroxide, either in pure or diluted form, can pose several risks, the main one being that it forms explosive mixtures upon contact with organic compounds. Highly concentrated hydrogen peroxide itself is unstable, and can then cause a boiling liquid expanding vapor explosion of the remaining liquid. Distillation of hydrogen peroxide at normal pressures is thus highly dangerous. It is also corrosive especially when concentrated but even domestic-strength solutions can cause irritation to the eyes, mucous membranes and skin. Swallowing hydrogen peroxide solutions is particularly dangerous, as decomposition in the stomach releases large quantities of gas leading to internal bleeding. Inhaling over 10% can cause severe pulmonary irritation.

Sodium percarbonate is a chemical, an adduct of sodium carbonate and hydrogen peroxide, with formula $2Na_2CO_3 \cdot 3H_2O_2$. It is a colorless, crystalline, hygroscopic and water-soluble solid. It is used in some eco-friendly cleaning products and as a laboratory source of

anhydrous hydrogen peroxide. Sodium percarbonate is more suitable than H_2O_2 as feed additives. Thus in this study, the effects of sodium percarbonate on inhibiting methane emission was studied *in vitro* and *in vivo*.

In vivo experiment was conducted in order to observe effects of feeding sodium percarbonate on methane emission from sheep. Based on the preliminary study the best level (2.0mM) was selected for further study.

MATERIALS AND METHOD

Four treatments *in vitro* were prepared as follows, 0.05mM, 0.1mM, and 0.5mM of hydrogen peroxide as final concentration in fermenting vessel and a control. The *in vitro* gas quantification system was used according to the protocol of Sar et al. (2005). Briefly, 10 g (dry matter) of dried milled hay and concentrate (70:30, w/w) was weighted into individual 1 L fermentation vessels together with 760 ml of a 4:1 volumetric mixture of carbon dioxide (CO_2)-saturated sterile artificial saliva (McDougall, 1948) and fresh rumen fluid inoculum from two fistulated Holstein cows (1:1, v/v). To start each experiment, 40 ml of each specific treatment was added to an individual vessel and the vessel was sealed to ensure anaerobic conditions. Fermentation was allowed to continue for 24 h at 39 °C. Gas output from each fermentation vessel was measured at 40 min intervals (for 10 min) by the auto infrared CH_4 (EXA IR, Yokogawa Electric Corporation, Tokyo, Japan) and CO_2 (Model RI-555, Riken Keiki Co. Ltd, Tokyo, Japan) analysers installed in the *in vitro* gas quantification system (Takasugi Seisakusho Co. Ltd, Tokyo, Japan). The pH and oxidation–reduction potential (ORP) of the fermentation medium were monitored in each vessel at 1-min intervals (TS mk-250, Takasugi-ss Co. Ltd, Japan). All data were pooled and stored to a computer through an interface with the analysers. At the end of each 24 h fermentation period, the contents of the fermentation vessels were discharged, and the vessels were thoroughly washed and autoclaved prior to the next fermentation. The fermentation vessels were then re-charged with fresh artificial saliva, rumen fluid inoculum and treatment to begin the next 24 h fermentation period. Each experiment was repeated three times on three separate days, with treatments assigned randomly to fermentation vessels each day.

Four sheep (62.1 ± 6.1 kg) were housed in metabolic crates equipped with a ventilated hood and allocated for use in a cross over designed respiration trial. The sheep were fed twice daily (8:00 and 16:00) at maintenance (55 g DM $kg^{0.75}$ BW per day) a diet of Kleingrass hay (DM; 87.9%, OM; 92.4%, CP; 13.2%, NDF; 66.4%, ADF; 32.6% and GE; 18.4 Mcal on DM basis) and formula feed (DM; 86.6%, OM; 96.7%, CP; 10.7%, NDF; 14.9%, ADF; 4.0% and GE; 18.47 Mcal on DM basis) which were mixed of 70:30 as dry matter basis with free access to water and a block of trace mineral salt (1232 mg Fe, 150 mg Cu, 25 mg Co, 500 mg Zn, 50 mg I, 15 mg Se and 382 mg Na per kg DM). Five gram of sodium percarbonate was added as treatments (2.0mM as final concentration of H_2O_2 in the rumen) and without sodium percarbonate as control. Each period lasted 14 days consisted 7 days for adaption, 5 days for collection of feces and urine, 2 days for gaseous measurement. The sheep were weighed prior to the beginning of each period.

CO_2 and CH_4 production were monitored continuously using a fully automated open-circuit respiratory hood system, as reported by Takahashi et al. (1998). The volume of CH_4 emitted from rumen fermentation was determined as the respiratory CH_4.

Air dried matter of mixed samples of refusals and feces collected for each collection period were prepared at 60˚C for 72 h in a forced-air oven for the analysis of digestibility. Feed, refusals and feces samples were ground to pass through a 1 mm sieve and stored in air-tight containers. Samples were analyzed for dry matter (DM; method 930.15) and organic matter

(OM; method 942.05) according to the AOAC procedures (1995). N determined by a Kjeldahl method (method 976.05; AOAC, 1995) using an electrical heating digester (DK 20, Actac, Tokyo, Japan) and an automatic distillation apparatus (UDK 130D, Actac, Tokyo, Japan). NDF of feed, feed refusal and fecal samples was analyzed according to the methods described by Van Soest *et al.* (1991) and ADF.

Data were analyzed by repeated measures analysis of variance according to the General Linear Models Procedures for a Latin square arrangement using SAS program (SAS Version 5.1.2600 Service Pack 3 Build 2600, SAS Institute Inc., Cary, NC, USA). Treatment means were compared using Tukey's multiple range tests. Statistical significance of differences was accepted at $P<0.05$.

RESULTS AND DISCUSSION

The results of *in vitro* experiment showed that a higher H_2O_2 concentration (0.1 and 0.5 mM) produced a lower ($P < 0.01$) cumulative *in vitro* CH_4 output by adding sodium percarbonate (Figure. 1). Dose dependent effect was observed from control till 0.1mM H_2O_2 concentration. The results from other observations indicated that, sodium percarbonate could inhibit methane production without negative impact on CO_2 production and volatile fatty acid (VFA) production *in vitro*. The use of 0.05mM of H_2O_2 was an optimum level for reducing methane emission without affecting other production parameters *in vitro*.

There was no significant difference in dry matter intake, so sodium percarbonate does not affect on palatability of feed. Figure. 2 shows the mitigating effect of dose of sodium percarbonate on CH_4 emission from rumen fermentation. Five gram of sodium percarbonate decrease CH_4 emission by 22.5% ($P<0.01$) *in vivo*. Based on the result, sodium percarbonate could inhibit methane emission from sheep, but this amount of sodium percarbonate had negative impact on rumen microbes. Therefore, it is necessary to consider the optimum amount of inclusion level of sodium percarbonate when used in ruminants diet without the harmful effects on digestibility of feed and rumen condition.

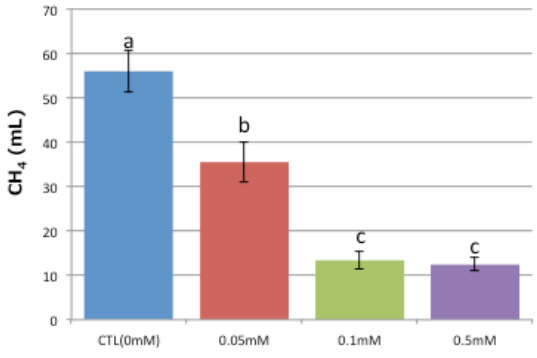

Figure 1. CH_4 production in vitro

(a,b,c: Different superscripts are significantly different at the 0.01.)

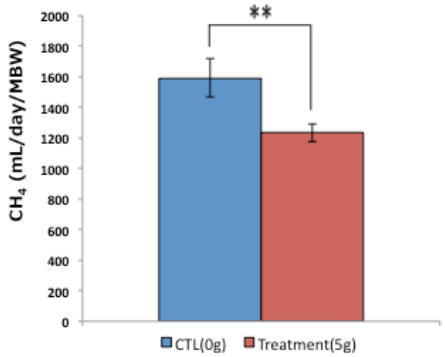

Figure 2. CH4 production with sheep

(**: $P<0.01$, MBW: Metabolic body weight, $kg^{0.75}$)

CONCLUSION

Based on the result, sodium percarbonate could inhibit methane emission with sheep, but this amount of sodium percarbonate had negative impact on rumen microbes.

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