BRIEF COMMUNICATION: Real time *in situ* measurement of rumen methane concentration in the rumen of cattle

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Keywords: rumen methane; rumen methane sensor; rumen pH.

INTRODUCTION

Methane dominates agricultural greenhouse gas emissions in New Zealand (Leslie *et al*., 2008). Research efforts in New Zealand to date have been directed toward prediction of emissions, most often using the rumen gas marker sulphur hexafluoride (SF6) (Lassey, et al., 1997) to predict methane emissions in grazing livestock. However, the errors associated with this method necessitate the use of multiple day sampling programmes and large numbers of individuals to overcome animal variability (Pinares-Patino & Clark, 2008). As a consequence, reporting of methane emission profiles is limited to 24 hour periods, as measurement of sub-diurnal intervals is effectively excluded. The use of closed chamber measurements of total gas emissions can increase confidence in methane output estimations. There are however some differences between these and SF6 techniques (McGinn et al., 2006). The measurement of rumen specific output in sub-diurnal intervals is still not reliable, and important information on energy transfer due to changes in animal behaviour can be lost (Lassey, 2008). The use of artificial rumen replacements such as in vitro continuous or batch culture fermenters has not adequately simulated the in vivo rumen measurements of hydrogen disappearance (McAllister & Newbold, 2008). Because of these limitations, assessment of methane emissions, including mitigation assessments, have necessarily used mean methane yields per day. Mitigation of methane emissions in grazing ruminants has not been successful. However, grass based production systems, particularly in the New Zealand dairy industry, are characterised by periods of greater and lesser intake and rumen activity (Gibbs *et al*., 2007). An alternative mitigation approach would be to target specific windows of rumen activity within the diurnal cycle of ruminants. However, assessment of that approach is severely limited by existing rumen methane estimation methods.

To date, little effort has been directed to understanding the rapid changes in environmental conditions within the rumen as a result of the metabolic activity of the rumen microorganisms, from which gas production arises. Consequently, predictions of rumen methane emissions are poor, as evidenced by a recent review reporting the strength of the correlation of predictors of methane yields as R^2 <0.2 (Machmüller & Clark, 2006). The challenge is to better understand how the patterns of rumen function alter the utilisation of nutrients and the fate of hydrogen ions in the short term, rather than simply estimating the gas production over extended intervals. A method of estimating rumen methane production across short intervals suitable to create sub-diurnal 'maps' of rumen activity should greatly improve our understanding in this area, and open up new approaches to mitigation of methane emissions.

This communication reports the use of a portable, *in-situ* rumen methane sensor in free grazing ruminants.

MATERIALS AND METHODS

A rumenally fistulated, 360 kg Holstein-Friesian steer was housed in an individual feeding pen. The steer was fed 5 kg dry matter (DM) of grass silage daily in split morning and afternoon meals. On Day 6 probes to measure *in-situ* pH, temperature (T), redox potential (RP) and the concentration of methane in solution were inserted into the steer's rumen. The methane sensing probe (MS) (Franantech, Lüneburg, Switzerland) was 25 cm in length and 4 cm in diameter, weighed 2 kg and was fitted with a 3 m cable. The battery powered unit was interfaced with a portable datalogger (GL500, Global Water Inc, California, USA) set to record methane concentration every one second. The sensor required calibration to an upper limit of detection prior to use. A likely upper limit of 350 µmol/L was selected. The T probe (Delta Ohm, TP47, Italy), pH probe (IJ44, Ionode Pty. Ltd., Brisbane, Australia), RP probe (IH30, Ionode Pty. Ltd., Brisbane, Australia) were deployed on a 1.5 kg open faced metal paddle, which in association with the MS probe were placed on the floor of the ventral sac of the rumen. The pH, T and RP probes were recorded every 15 seconds to a datalogger (Delta Ohm, HD 2105.2, Italy). Recorded values for the period from 1 hour before to 3 hours after feeding were used in subsequent statistical analysis.

FIGURE 1: The effect of feeding 5 kg dry matter of grass silage on the *in situ* temperature, pH, soluble methane concentration and redox potential of the rumen contents of a Holstein-Friesian steer. For each parameter displayed, each data point represents the mean value with standard deviation of 10 minutes of recorded values.

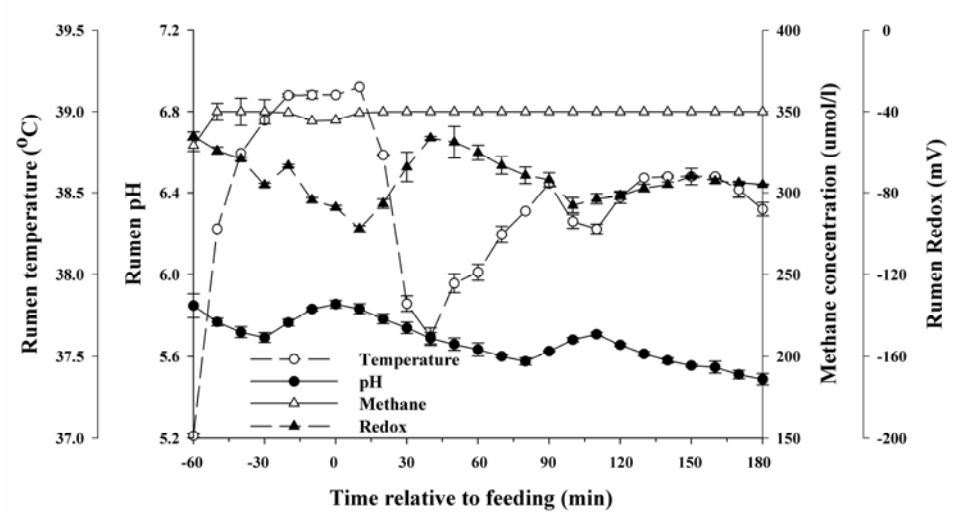
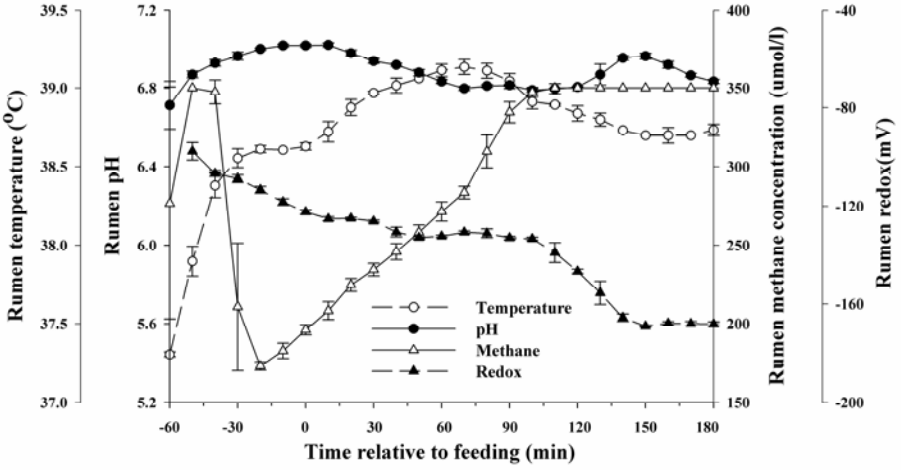


FIGURE 2: The effect of feeding 1 kg dry matter barley straw on the *in situ* temperature, pH, soluble methane concentration and redox potential of the rumen contents of a Holstein-Friesian steer. For each parameter displayed, each data point represents the mean value with standard deviation of 10 minutes of recorded values.



The steer was removed from the crate and grazed on pasture for 14 days before being fed 5 kg DM barley straw daily for a further 3 days outdoors and replaced in the individual feeding pen. The barley straw was removed at 17:00 h. The next morning the measurements described for Day 6 above were repeated, but only 1 kg DM of barley straw was fed.

The recorded pH, T, RP and methane concentration values were compressed to a mean value for every 10 minute interval across the recording periods for each parameter. These mean values were used in linear regression analysis (SPSS, 2007) to determine the correlation of each parameter and the combination of all parameters to the recorded methane concentration.

RESULTS

The rumen pH, T, RP and methane concentrations recorded during Day 6 are displayed in Figure 1. The results showed the methane probe was quickly saturated to the calibrated upper detection limit of 350 µmol/L. Rumen pH was below 5.9 for the recording period, ranging from 5.55 to 5.85. T declined after feeding from the pre-treatment value, to a nadir 45 minutes after feeding of 37.5°C, then increased to remain between 38 and 38.5°C for the rest of the period. The RP was below -100 mV for the entire recorded period, ranging from -60 mV to -95mV. The constant value of 350 µmol/L of methane recorded across the period

prevented any comparisons of methane concentration with pH, T or RP.

In the second recording period (Figure 2) the recorded methane concentration immediately prior to feeding was considerably lower at 180 to 200 µmol/L than that of Day 6 (Figure 1), and did not reach sensor saturation of 350 µmol/L for approximately two hours. There was a consistent increase in recorded methane concentration of approximately 10 to 25 µmol/L every 15 minutes over this period. Rumen pH was consistently higher than that recorded on Day 6, remaining between 6.7 and 7.0. Rumen T was between 38.5 and 39.1^{o}C from feeding until the conclusion, while RP declined across the recording period from -100 mV to -165 mV.

Rumen methane concentration was moderately and negatively correlated to RP (R^2 = -0.582, P >0.05) and also to the pH (R^2 = -0.528, P >0.05), but there was no significant correlation to rumen T (R^2 = 0.004, P <0.05). A linear regression analysis showed pH and RP together could explain 61.8% of the variation in rumen methane concentration (Equation 1). However, if the recorded values for the period from 60 to 30 minutes before feeding were excluded as a stabilisation phase for the sensor, a higher correlation between rumen methane concentration and pH (-0.681, P >0.001), RP (-0.813, P >0.001) and rumen T (0.31, P >0.001) was observed. Equation 2 demonstrates pH and RP explain up to 91.2% of the variation in recorded methane concentration for the abbreviated period.

Equation 1

Rumen methane concentration (µmol/L) = 3,165 (± 31) - 1.41 (± -0.02) RP – 444 (± 4) pH
R^2 = 61.8%

Equation 2

Rumen methane concentration (µmol/L) = 2,689 (± 15) - 2.45 (± 0.01) RP – 398 (± 2) pH
R^2 = 91.2%

DISCUSSION

This experiment was designed to assess if a portable methane sensor could be used in a novel application in the rumen to measure changes in methane concentration of rumen fluid across short intervals in real time. The methane sensor appears capable of ready adaption to use *in situ* in bovine rumens, and the entire integrated system developed for this project of sensor, power supply and data recording, is readily portable for use in free grazing cattle. This sensor measured changes in rumen methane solutions of less than 25 µmol/L across 15 minute intervals, which demonstrates it is sensitive to small shifts in methane concentrations across short intervals to enable effective monitoring of rumen methane production in sub-diurnal measurement periods. This is a valuable new tool for rumen methane concentration assessment, and opens up opportunities for fresh approaches to the study of rumen metabolism.

In addition, the significant correlation of rumen pH and RP to methane concentration in this pilot study suggests further research in this area is warranted. There is a recent increase in published work in rumen pH assessment in real time, *in situ* applications (Dohme *et al*., 2008). Accurate 'mapping' of the rumen environment by indwelling monitor technology is now possible. There is an obvious advantage in using detailed, accurate measurement of the rumen environment to further understand of rumen methane production, to improve the very poor predictive efficacy of methane production to date.

Methane concentration in the rumen liquor *in vivo* has not been reported before, and so it was difficult in the design of this study to determine the range or maximal concentration likely to be observed. Duan and Mao (2006) provide values for saturated methane solubility in various ionic solutions at physiologic temperatures and pressure of between 870 and 1,110 µmol/L. This represents a maximal value of methane concentration in rumen fluid approximately threefold above the calibrated upper detection limit used in this experiment. The results in this experiment show that even at relatively high rumen pH, the methane in solution is above approximately half saturation in the ventral sac within a few hours of even a small meal of low digestibility forage (Figure 2), and at or above that level even between meals of higher digestibility forage (Figure 1). This is a surprising finding in light of what is understood of rumen methane production. Murray *et al*. (1976) suggest that methane production in the rumen is related to the concentration in the gas phase. As a result of the high dissociation constant of methane of pKa = 56 at the pH range observed in a cattle rumen, it was estimated that most of the methane would readily escape the liquid phase and accumulate in the gas phase of the rumen or be eructated rather than accumulate to saturation in the rumen liquor. However, in this experiment methane concentrations above 160 µmol/L were recorded even before the meal, and despite the steer being fed only barley straw at a very low intake rate, which suggests significant accumulation of methane in the liquid phase.

CONCLUSIONS

This preliminary study suggests that it is possible to use a MS probe to measure the methane concentration in the rumen contents of cattle in real

time and *in situ*. In future studies the MS probe will be calibrated to record higher methane concentrations than used in this study. Our analysis also suggests a correlation between methane concentration and rumen pH, T and RP, which warrants further research. These results also add further evidence of the complexity of the metabolic pathways for methane emissions in cattle, which could be relevant to a better understanding of the known differences between rumen marker and chamber methods for methane yield estimation, and the observed variability between diets and animals.

REFERENCES

Dohme, F.; DeVries, T.J.; Beauchemin, K.A. 2008: Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk fro developing acidosis ruminal pH. *Journal of dairy science* **91**: 3554-3567.

Duan, Z.; Mao, S. 2006: A thermodynamic model for calculating methane solubility, density and gas phase composition of methane-bearing aqueous fluids from 273 to 523K and from 1 to 2000 bar. *Geochimica et cosmochimica acta* **70**: 3369-3386.

Gibbs, J.; Laporte-Uribe, J.; Trotter, C.; Noel, J. 2007: Rumen function and lameness in pasture based dairy cows of the South Island of New Zealand. *Journal of dairy science* **90**: 655.

Lassey, K.R. 2008: Livestock methane emission and its perspective in the global methane cycle. *Australian journal of experimental agriculture* **48**: 114-118.

Lassey, K.R.; Ulyatt, M.J.; Martin, R.J.; Walker, C.F.; David Shelton, I. 1997: Methane emissions measured directly from grazing livestock in New Zealand. *Atmospheric environment* **31**: 2905-2914.

Leslie, M.; Aspin, M., Clark, H. 2008: Greenhouse gas emissions from New Zealand agriculture: issues, perspectives and industry response. *Australian journal of experimental agriculture* **48**: 1-5.

Machmüller, A.; Clark, H. 2006: First results of a meta-analysis of the methane emission data of New Zealand ruminants. *International congress series* **1293**: 54-57.

McAllister, T.A.; Newbold, C.J. 2008: Redirecting rumen fermentation to reduce methanogenesis. *Australian journal of experimental agriculture* **48**: 7-13.

McGinn, S.M.; Beauchemin, K.A.; Iwaasa, A.D.; McAllister, T.A. 2006: Assessment of the sulfur hexafluoride (SF6) tracer technique for measuring enteric methane emissions from cattle. *Journal of environmental quality* **35**: 1686-1691.

Murray, R.M.; Bryant, A.M.; Leng, R.A. 1976: Rates of production of methane in the rumen and large intestine of sheep. *British journal of nutrition* **36**: 1-14.

Pinares-Patino, C.S.; Clark, H. 2008: Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. *Australian journal of experimental agriculture* **48**: 223-229.

SPSS, 2007: SPSS 17.0. SPSS Inc., Chicago, Illinois, USA.