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Blood methane concentration as a marker for bovine greenhouse gas emissions

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Abstract

Methane blood concentration and Ostwald solubility coefficients were measured at 39^{o}C on dissolved gas samples stored into Exetainer tubes at 4^{0}C for 20 day. The blood of grazing steers had a mean value of 15.6 ± 9.84 ng/mL and 2.4 ± 2.25 for methane blood concentration and Ostwald solubility coefficient, respectively. When measured across breeds, methane blood concentration was similar between Brahman (18.1 ± 5.20) and Belmont red composite (14.6 ± 3.66) steers, while no differences were detected among low (17.3 ± 4.12), medium (19.7 ± 4.62) or high (12.0 ± 4.41) digestibility profile groups. There were not digestibility treatment effects upon methane Ostwald solubility coefficients, but higher (P < 0.05) values were in Belmont red composite than in Brahman cattle. Irrespective of breed and digestive profile, there was a significant (P < 0.05) negative relationship between methane blood concentration and platelet (- 0.55) or total protein contents (- 0.52) in the body fluid. It was concluded that although no consistent differences existed in methane blood concentration among experimental groups, multiple measurements on samples of identical origin will contribute to a better standardization of the blood technique. This will also help to understand the physiological variability of methane concentration and solubility coefficients in blood of Northern beef production systems. Therefore, the use of the methane blood technique is recommended to assess the association between those blood variables and short and total methane emissions measured currently by open circuit calorimetry at Lansdown Research Station.

Executive Summary

Methane (CH_4) emissions from cattle grazing pastures are difficult to measure if the objective is to assess individual emissions and the animal to aminal variability in emissions. Current techniques are expensive and not suited to large numbers of animals.

It is known that methane is diffused into the blood stream. This project was conducted to determine if there was a reliable relationship between blood methane concentration and methane emissions measured using open circuit calorimetry.

The first phase considered the methodology for blood collection and analysis and the technique for assessing methane solubility in blood. The second phase (incomplete) comprises two studies, one with dairy cattle and one with tropical beef cattle. In both studies htere were a range of treatments to provide a rigorous test of the relationship and dry matter intake ranged from around1.5 to 3% of body weight, depending on the class of animal.

Results have shown that the method works and blood methane concentration and solubility have been successfully measured. Technical difficulties have delayed the conduct and analysis of ht second phase of the work.

Contents

Page

1. Background

Measurement of methane emissions from cattle is expensive and difficult to achieve without seriously impacting the normal behavioural responses of the animal. Calorimeters can successfully determine methane emissions from known amounts of offered feeds, but this system is not practical for large numbers of animals, as required for example, in determining heritability. Nor can it replicate the grazing environment where animals are free to select different dietary components and exhibit normal behaviours. Mean methane emissions from groups of cattle can be obtained by using laser technique (B.CCH 1063) but this method does not allow for the measurement of individual animal variation. Current research is developing an indwelling rumen bolus that can measure individual methane concentration in the rumen of grazing animals. This promising method represents a step forward in estimating individual methane emissions of grazing animals. However, it does involve the dosing of animals with an expensive sensor that cannot easily be retrieved and furthermore the relationship between the concentrations of gases measured by the sensors and the magnitude of methane emissions is still to be understood.

Considering the limitations of all the above (and other) techniques, this project aimed to investigate the possibility that a simple blood assay could be developed that provided a proxy for methane emissions from cattle. Methane is formed anaerobically in the rumen and the caecum as metabolic reduction of carbon dioxide and hydrogen during the fermentation of plant structural carbohydrates. The gas is mainly eructed, breath expired and lost in the flatus. The fermented gas is also absorbed into the blood stream. The key question addressed in this project is the application and validation of a novel blood analysis to measure methane on individual basis (Ramírez Restrepo et al., 2010). The technique was developed using animals in grazing conditions, but has not previously been validated against open-circuit calorimetry.

2. Project Objectives

The first objective of this study was to standardize the blood analysis technique. The second objective was to determine the relationship between blood CH_4 concentration and CH_4 emissions measured by calorimetry at CSIRO Lansdown Research Station using tropical beef breeds and at DPI Victoria Ellenbank Research Station using dairy cattle. Thus the method was tested for a range of cattle types and intakes and across a number (6) treatments. The research is based on the following hypotheses: (1) methane jugular blood concentration is similar to the gas concentration entering the lungs that represents gas absorption from the rumen and the lower gastrointestinal tract; and 2) the blood CH_4 gas concentration profile is related to confined measurements on whole-animal calorimetric measurements.

The key question addressed in this project is the application and validation of a novel blood analysis to measure methane on individual basis (Ramírez Restrepo et al., 2010). The technique was developed using animals in grazing conditions, but has not previously been validated against open-circuit calorimetry. The project will associate skills from CSIRO Livestock Industries (CLI) and expand its commitment to developing environmentally sustainable and efficient ruminant production systems and contribute to the strategy of improved animal selection for low emitting populations of cattle

3. Methodology

3.1 Developing the laboratory techniques

Jugular blood was collected from 18 two-year old grazing steers at Lansdown research Station (i.e., Brahman vs. *Bos taurus* composite) balanced by low, medium and high *in vivo* DM and OM digestibility profiles and from 4 lactating Friesian dairy cows at the DPI Victoria Ellenbank facility (Peter Moate). These were used for calibration of the blood methane technique

Relative to the previous research, the present work analysed equilibrated gases that were kept into Exetainer tubes (Labco Ltd, Buckinghamshire, UK) at 4^{o}C for 20 days due to uncontrolled limitations in the lab. Simultaneous measurement of methane blood concentration and Ostwald solubility coefficients were performed by gas chromatography as described previously (Ramírez-Restrepo et al., 2010). Briefly, the instrumentation and injection phase can be summarized as follows.

After refrigeration, samples were allowed to reach room temperature (24^{o}C) for one hour. Samples (5 ml) from the gas phase were anaerobically drawn in a 5 ml gas-tight syringe (SGE, Analytical Science Pty Ltd, Rigwood, Victoria, Australia) and injected into a 2014AF gas chromatograph (GC; Shimadzu, Tokyo, Japan) via a 2-ml constant-volume inlet loop for adequate flushing and filling. The GC system was configured with flame ionization (FI), electron capture (EC) and thermal conductivity detectors and a GC Solutions V2.3 software. Methane was separated on a ShinCarbon ST Micropacked column (100/120 mesh, 2m, 1/16in, OD 1.0 mm ID; Restek Corporation, Bellefonte, PA, USA) using purified Nitrogen (999: 1000 (v/v); BOC, Sydney, NSW, Australia) as the carrier gas.

Determination of Ostwald solubility coefficients and blood methane concentrations were performed by comparison of the peak area of the known standard (CH_4 20.2 ± 0.50 parts per million (ppm), BOC, Sydney, NSW, Australia) versus that of the unknown samples.

Mean values and coefficients of variation (CV) for blood methane concentration and Ostwald solubility coefficient were assessed using the MEANS procedure (SAS, 2008). Differences in blood estimates of methane concentration and the solubility coefficient of the gas were assessed using the MIXED procedure of Statistical Analysis System (SAS, 2008). The linear model included the fixed effects of breed, treatment (i.e., digestibility profiles) and the interaction between breed and treatment. Live weight was used as a covariate in the analysis.

Correlations among methane blood concentration and blood background levels were analysed using the CORR procedure (SAS, 2008).

3.2 Correlation between blood methane concentration and methane emissions measured in open circuit calorimeters

This phase of the trial has been delayed due to ongoing problems with the gas chromatograph and the need to successfully calibrate the new methane chambers at Lansdown. However the initial scope of the study has been to include 3 successive samplings of blood from lactating Friesian dairy cows at the DPI Victoria Ellenbank facility (Peter Moate). Essentially it involved the collection of jugular blood samples from cattle during or just after methane measurements in calorimeters.

The Ellenbank study was a cross-over design trial with three treatments and three periods. The treatments were designed to elicit different methane emissions. Blood samples have been collected and await analysis. Data will be correlated with the methane emissions from the dairy cows collected one day prior to the blood sampling.

The Lansdown study is an incomplete Latin square design with two breed types (Brahman and a tropically adapted composite breed (Belmont red). Within each breed two animals have been selected at each of three digestibility categories. Digestibility was estimated in the cattle during a previous grazing study by faecal NIRS methodology.

The Ellenbank study will be analysed for the effect of treatment using standard techniques employed at that centre. Treatment, period, animal breed and digestibility category effects for the Lansdown trial will be determined by ANOVA methods using SAS.
The correlation between calorimeter and blood methane values will be determined by regression analysis. Blood methane concentration will be converted to the same units and tha calorimeter data using this relationship. Secondly the agreement between the derived values from blood and the values from calorimeters will be tested using the mean prediction error (MPE). This is calculated by

summing the squares of differences between observed and estimated values, dividing by n and taking the square root of this quotient.

4. Results & discussion

4.1 Developing the laboratory techniques

The blood contained similar measurable concentrations of methane across breeds or digestibility profile groups (Table 1). Belmont red steers had lower ($P < 0.05$) Ostwald solubility coefficients than Brahman steers, but digestibility profile groups had similar solubility coefficient values. Across all treatments, the overall mean coefficient of variation for blood methane concentration was much lower than those for the Ostwald solubility coefficient.

Mean cell volume, platelet, total blood protein and cholesterol values were negatively related to methane blood concentration in Brahman and Belmont red composite steers, while the negative interaction between methane blood concentration and platelet counts is similar in steers across all digestibility profile groups (Table 2). Irrespective of breed and digestive profile, blood concentration of total protein (- 0.52) and platelet counts (- 0.55) were negatively ($P < 0.05$) related to the concentration of methane in the blood.

The objectives of this experiment were firstly to standardize the methane blood concentration technique using blood samples from grazing cattle, and to assess the association between methane blood concentration profiles and short and total methane emissions measured by open circuit calorimetriy. The second objective will be conducted over the next three months. To date, the most significant finding was that to the best of the author knowledge, this is the first time in which methane concentration and Ostwald solubility coefficients has been quantified in blood of beef cattle at body temperature in the tropics (Finch, 1986).

Under these circumstances, the present investigation also showed that methane blood concentration is associated with some haematological parameters. However, as the blood methane concentration depends on the Ostwald gas solubility coefficient, values obtained in this preliminary study must be interpreted with caution. Inherent variability between samples are related to total extraction of the gas from the liquid phase, reduced loss of gases, accurate,

sample for injection, detector sensitivity and correct records of temperature and pressure at the time of measurements (Meyer, 1978; Lango et al., 1996).

In summary, despite some limitations in the lab and the late commission of calorimetric chambers, it is recognized that under conventional operating conditions, of particular interest is to elucidate the relationship between short and total methane emissions in chambers and methane concentration profiles over a 24-h in normal blood. Indoor investigation at Lansdown Research Station is currently underway.

4.2 Correlation between blood methane concentration and methane emissions measured in open circuit calorimeters

In May 02 2011 a new gas chromatograph was purchased with the capability to measure methane concentration in blood. This piece of equipment is still not working property after more than 12 months of intensive attention by both the company technicians and CSIRO staff. We have had intermittent periods of successful runs, which has allowed analysis for the first phase of the trial. In addition this equipment is housed in a new facility and the gas lines which bring gases to the GC from an outside storage area were not installed correctly and leak. This has resulted in frequent down-times and excessive losses of gases. Consequently the project is behind schedule in regard to blood methane analysis.

The newly constructed calorimeters at Lansdown are still undergoing testing and refinement. The Lansdown trial was set to run in may 2012, however just prior to this, it was discovered that there were problems with the air flow rates and turn-over times. This is currently being rectified by the installation company and we hope to run the study in July.

5 Conclusions

Technical difficulties have delayed progress on the final stages of this project. However, the work will be completed after the end of the project with CSIRO support. Nevertheless, the initial phase of this work has successfully demonstrated for the first time that it is possible to measure methane concentration and solubility in the blood of cattle across a range of conditions. Data on the relationship between blood methane and eructed methane will be available within 6 months.

Assuming such a relationship can be developed with a known amount of uncertainty associated with it, the technique will be a significant breakthrough. The ability to use blood methane concentration as a proxy for methane emissions represents a cheap and simple technique that can be used on large numbers of cattle under a range of conditions.

Provided this relationship can be established future work should concentrate on developing a crush-side blood test that can be applied in the field.

Table 1
Live weight (LW), dry matter intake (DMI) and blood gas chromatography in grazing Brahman and Belmont red composite steers grouped by digestibility coefficients (g/g) at Lansdown Research Station on the east coast of North Queensland, Australia. Data area expressed as least square mean values ± SEM.

	Breed		Digestibility profile		
	Brahman	Belmont red	Low	Medium	High
n	10	8	6	6	6
Live weight (kg)	402.4 ± 10.4 a	437.6 ± 9.26 b	422.6 ± 11.8	422.6 ± 11.8	414.7 ± 12.5
Intake[†]					
DMI (kg/day)	7.1 ± 0.18 a	7.6 ± 0.16 b	7.12 ± 0.21	7.70 ± 0.21	7.35 ± 0.22
Nutrient digestibility[†]					
DM	0.5809 ± 0.0026	0.5833 ± 0.0023	0.5773 ± 0.0030 a	0.5793 ± 0.0030 a	0.5898 ± 0.0031 b
OM	0.5918 ± 0.0012 a	0.6002 ± 0.0010 c	0.5842 ± 0.0013 a	0.5951 ± 0.0013 b	0.6088 ± 0.0014 c
n	9	8	6	5	6
Methane					
Blood concentration (ng/ml)	18.1 ± 5.20	14.6 ± 3.66	17.3 ± 4.12	19.7 ± 4.62	12.0 ± 4.41
Ostwald solubility coefficient	1.2 ± 0.71 a	3.4 ± 0.57 b	2.0 ± 0.64	2.0 ± 0.72	2.9 ± 0.69

n: Experimental animals.

[†] Predicted by faecal near infrared reflectance spectroscopy (NIRS); [‡] Adjusted to equal body weight at the time of sampling.

Means between columns within each variable with different letter differ (a, b; $P < 0.05$), (a, b, c; $P<0.001$). NS, no significance.

All blood samples were analysed in duplicate.

Table 2
Differential correlation coefficients (*r*) between methane blood concentration (ng/ml) and blood composition in grazing steers over the late spring season of 2011 at Lansdown Research Station on the North East coast of Queensland, Australia.

	Breed				Digestibility profile					
	Brahman		Belmont red		Low		Medium		High	
n	10		7		6		5		6	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Red blood cells (RBC; IU*10^{12}/L) †	0.50	NS	0.09	NS	-0.13	NS	0.65	NS	-0.06	NS
Haemoglobin (HB; g/L) †	0.07	NS	0.18	NS	0.01	NS	0.09	NS	0.49	NS
Haematocrit (HCT; %)†	0.36	NS	0.01	NS	-0.28	NS	0.28	NS	0.41	NS
Mean cell volume (MCV; fL) †	- 0.44	NS	-0.25	NS	-0.15	NS	-0.71	NS	0.19	NS
Mean cell haemoglobin (MCH; pg) †	-0.53	NS	0.29	NS	0.25	NS	-0.84	0.07	0.55	NS
Mean cell haemoglobin concentration (g/L)†	-0.30	NS	0.36	NS	0.47	NS	-0.62	NS	0.49	NS
Platelets (x 10^9 cells/L) †	-0.51	NS	-0.58	0.08	-0.74	0.09	-0.27	NS	-0.51	NS
Total blood protein (g/L) †	-0.80	0.05	-0.49	NS	-0.66	NS	-0.71	NS	0.29	NS
Cholesterol (mmol/L) †	-0.35	NS	-0.39	NS	-0.84	0.05	0.21	NS	0.26	NS
White blood cells (IU*10^9/L)†	0.30	NS	-0.21	NS	0.29	NS	-0.42	NS	-0.16	NS

n: Experimental animals.
† Adjusted to equal live weight at the time of bleeding.

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