Final report

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Can multiple short-term measures of methane be used to quantify daily methane emissions: beef cattle?

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EXECUTIVE SUMMARY

- The GreenFeed Emissions Monitoring (GEM) devices are unique patented tools which use a 'bait station' to attract individual cattle to a small feeding booth in which their CO2 and CH_4 emissions are measured over 3-5 minutes, 5-10 times per day.
- They can be used to determine total CH_4 production, total CO_2 production, and also differentiate CO_2 arising from mammalian respiration, from that arising from rumen fermentation.
- This study evaluated a GEM unit using supplement and a GEM unit using water as the baits.
- With appropriate filtering, the estimate of daily emissions from the two units was within 1% of each other.
- A comparison of GEM measured emissions and Calorimeter measured emissions from cattle was slightly confounded by reduced intake on days when cattle were in chambers, however the methane yields (g CH4/kg DMI) were very similar between the two systems.
- Emissions form individual animals were remarkably stable over time (CV < 6%)
- Recovery of a carbon dioxide pulse (as a test gas) through the GEM unit was 99%
- We are convinced that the GEM units can, with appropriate control, provide an accurate measure of daily methane emissions period, based on our and New Zealand (not included) data.
- We have had continuous assistance during these studies by the GEM manufacture team.
- We have also made substantial progress in how to introduce animals to the GEM units and get the animals using them well.
- However, there are some weak points in the GEM armour that need to be improved.
    - Large (daily) dependence on manufactures to process and quality check data. While this is helpful in this testing stage, it is not realistic or helpful if such support was found to be essential for data to be collected into the future. We have received continuous assurance that they will equip us for independent operation…but we are not there yet.
    - A large number of hardware failures/replacements that have needed to be 'talked through' and use software patches from USA. While we expect these are just teething troubles with a new unit and have come to an end, IF such intensive input were an ongoing need would preclude use of the units in all but the most managed environments in which a technician is always on hand.
    - An intensive calibration schedule of sensors is required that again mean the current GEM units are not devices that can be parked in the paddock, switched on (to 12v power) and abandoned with an expectation that data will appear in your email every day.
    - Low supplement holding capacity and general construction standard that is less robust than would be required for use in harsh environments (eg. exposed cabling, heavy use of plastics and silicon sealant)
    - We have held discussions with C-Lock about increasing the level of auto-calibration to allow use in more remote environments and in the absence of skilled technical staff.
- In summary, the GEM device have brought new possibilities for in-paddock emissions measurement that will provide a powerful capability for validating emissions and mitigation

levels, while also providing a valuable tool for feed efficiency research.

PRINCIPLES OF THE GREENFEED TECHNOLOGY

The device known as a GreenFeed unit or GEM (Greenfeed emission monitoring unit) is a patented technology developed by C-Lock Inc. in South Dakota USA (US Patent 7966971, others pending). It measures the enteric emissions of methane and carbon dioxide breathed and eructated by ruminants when they voluntarily place their head in a feeding hood for 3-5 minutes during which time a metered amount of supplement is slowly delivered to them as an attractant. Methane production is measured continually while animals have their head in the stall, by measuring the methane concentration in air drawn through the hood during this time (Figure 1). The rapid response sensors detect individual eructation events (about every 30 seconds) and a data stream of gas concentrations is relayed by wireless internet every 10 minutes to C-lock USA and the processed results returned overnight to Australia by email. The system has multiple internal checks but requires calibration of gas sensors at least weekly and this is manual. The electronics is 12v and so can be powered by a solar array (UNE do not have this). A 'master class' in GEM use will be run in USA in late 2012 and we hope to have a technician attend.

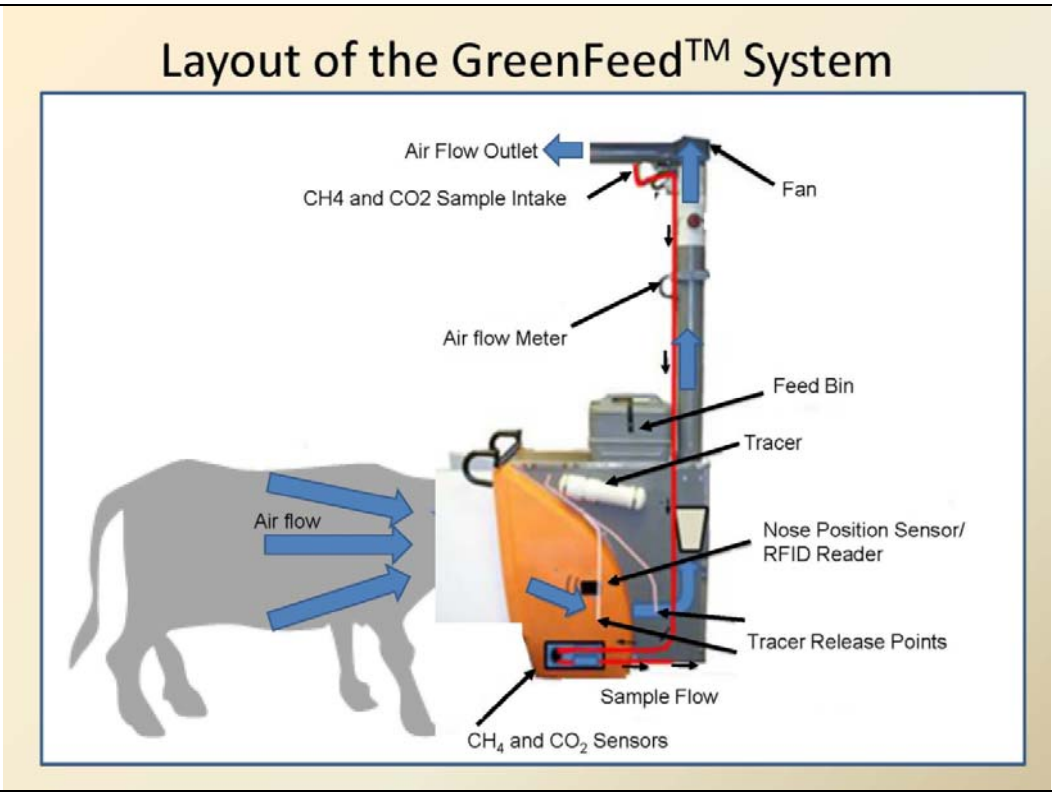


Figure 1. Key components in the GreenFeed Emissions Monitoring unit

Experiment 1: An evaluation of short term methane production measures as indicators of daily methane production by cattle.

Introduction:

The daily production of enteric methane from cattle has been determined in fixed feeding environments using a suite of calorimetry (Blaxter & Wainman, 1964), mask and tracer dilution methods (Hegarty et al., 2007). In the grazing environment these emission have been measured on individual and groups of cattle using SF_6 tracer and open path methods respectively (Pinares-Patino et al., 2003; Jones et al., 2011). More recently, the recognition that short periods of measurement of emissions explain large proportions of daily emissions, has led to the testing and development of methods for measuring emissions over short periods (Robinson et al., 2010; Goopy et al., 2011). One such technology for making repeated short term measurements of emissions from grazing cattle is the Greenfeed Emission Monitoring (GEM) system. This technology allows measurement of 5-10 periods of 3-5 minutes of emission per day while animals voluntarily consume a slowly delivered supplement, the supply of which is computer controlled. This report investigates some of the attributes of GEM units (one that delivers pellets as an attractant and one that delivers water), together with assessing limits to their use and ways to overcome impediments to use.

Materials and Methods (*note this section is as reported in Milestone 4*)

Animals and housing

Six shorthorn female cattle of varying age and ranging in body weight from 314 to 680kg (mean = 477kg) were group-housed in an open pen (12m x 10m) in an open barn (Figure 1), with a comparably sized external spelling area. Cattle were introduced and adapted to the diet delivered through the Ruddweigh automated feeder, and introduced to the GEM supplement dispenser and to the GEM water-dispenser for 3 weeks.

Feed and feed intake

An oaten chaff/lucerne chaff blend (ME 9.2 MJ/kgDM; 11.9%CP) was provided *ad-libitum* through a "Ruddweigh" feed dispenser fitted with RFID identification. This ensured that each meal of each individual animal was recorded (Bindon 2001). The accuracy of feed recording was checked against the known weight of feed added to the unit daily. Because knowledge of feed intake will be desirable in future grazing applications of GEM units for methane emission yield studies, the digesta markers chromic oxide and ytterbium acetate were incorporated into the pellets delivered through the GEM supplement unit as described below:

Figure 2. Interior pen showing the GEM water dispenser (nearest orange device), GEM supplement dispenser (distant orange device) and Ruddweigh ration dispenser (green at far end) aligned along the edge of the pen. Each unit has a 2.4m access raceway to the unit to ensure only one animal access at a time so that multiple RFID tags do not disrupt the electronics.

<u>GEM supplement unit</u>

A custom pellet based on hammer-milled rolled barley and lucerne chaff was prepared for use, assuming a daily allocation of 1.5kg supplement/head. Chromic oxide (Cr) and ytterbium acetate tetrahydrate (Yb) were included in sufficient quantity to provide approximately 1000mg Cr/d and 200mg Yb/d per head. The GEM unit delivers the supplement via a rotating cup and the number of drops of pellet per feeding event, together with the time delay between feeding events can be controlled so that the number and duration of feeding events is optimised for estimation of methane production. The repeatability and mass of supplement delivered per drop was calibrated and found very repeatable (CV~3%). The supplement containing pellets were exhaustively mixed in a tub mixer prior to pelting to ensure uniform marker concentration. Samples of pellets were taken from the GEM supplement hopper daily to confirm the uniformity of the marker concentration over time.

<u>GEM water unit</u>

The unit was the first of its type designed and built. Like the GEM-supplement unit, as well as measuring methane emissions the intent was to use the unit to allow estimation of intake by dispensing a (soluble) digesta marker (CoEDTA) in the water, with the unit recording the water intake of each animal at each drinking event. However, the balance of water flow-rate and time taken for animals to drink meant that we were unable to accurately measure water intake from the individual cattle in this study, so no CoEDTA was delivered through the water unit. A new low-volume flow meter has been supplied but was not available when the need for it became apparent during the study.

Experimental timetable

Once cattle were accustomed to both the GEM supplement and water units and to delivery of the main ration through the Ruddweigh feeder, a background faecal sample was collected from the rectum and a 24h collection of exhaled gas was made into an evacuated canister mounted on the back of the cattle (Hegarty et al., 2007;Fig 2). Supplement containing Cr and Yb markers as described was then placed in the GEM supplement unit and cattle received this marker-containing supplement for 7d to allow faecal concentrations to plateau. A calibrated SF_6 releasing high-flow permeation tube was then inserted by a balling gun into the rumen of each animal. A series of six consecutive 3d measurement periods then commenced. The same program was followed in each period as described below.

At approximately 10am, a faecal sample (120g) was collected as cattle were in or approaching the cattle handling area and immediately frozen (-18^{o}C). A halter fitted with two interconnected air sampling points above the nostrils was placed on each animal at the start of the first period and left on the animal for the duration for the study. An evacuated (4L) aluminium canister was mounted on the back of each animal and connected to the air sample line on the nose by a 1/8th diameter tube with in-line filter and flow restrictor (0.6-0.8ml/min flow). At approximately 11am, cattle were the returned to the pen where GEM and Ruddweigh units were installed. Cattle were left undisturbed for 47h and then moved to the cattle handling area where a faecal sample was collected and the gas collection canister was removed. These tasks were completed within 1h and cattle moved into individual respiration chambers. At 11am feed was provided to cattle in their individual respiration chambers and the door sealed to allow emission measurements to commence. The amount of feed offered to each animal was the average daily intake over the preceding 48h, based on their consumption recorded by the Ruddweigh feeder. In addition, a quantity of marker-containing supplement pellets equal to their average daily intake over the preceding 48h was mixed with the main ration, which was provided as a single daily meal in a 120L feed bin.

Figure 3. Heifer with SF_6 collection canister mounted on her back while accessing the GEM water dispenser

Methane measurement

Respiration chambers

The respiration chambers (5) used for methane measurement were of polycarbonate construction and open circuit design, with a water seal onto the concrete floor. Approximately 1.5m^3/ of external air was drawn into each chamber per minute from a common intake with individual ducts (Figure 3). A continuous subsample of air (2L/min) was drawn from the exhaust of each chamber, pumped through a refrigerated dryer and passed through a multiplexer. The multiplexer was programed to direct sample from each chamber and from the external air into a Servomex gas analyser every 6 minutes (50s purge, 10s measurement), with the concentrations of methane, carbon dioxide and oxygen being recorded on proprietary software (AZCO Holdings). The gas analyser was calibrated prior to cattle entering the chambers, and calibration gases used to check for drift after cattle were removed. A gas standard was also used to check for leaks though the gas collection and handling system.

Figure 4. Open circuit cattle respiration chambers used in this study. Cattle were fed a matching intake to what they had consumed over the previous 2d in the yards, however there were refusals in most cases which will complicate the chamber:GEM comparsions.

ASCO sniffer

A custom made device for measuring the concentration of methane and CO_2 in air within the feed bay of the Ruddweigh feeder was to be used to determine and record these gas concentrations. Unfortunately, problems with matching this data with the RFID tag read reading meant no data from this device was collected.

Greenfeed methane emission monitoring (GEM) units

Before the first measurement period commenced, the air flow was determined across the cross-section of the exhaust towers as required. Both Greenfeed units were managed the same way in the 3d experimental periods used. At commencement of a period, the sensors in the units were calibrated using nitrogen as a zero gas, and separate methane and CO_2 standards to span the instruments. Once the entire study was complete, the weight of cylinders of pure CO_2 was measured as CO_2 was released into the mouth of the greenfeed units. This data was used to confirm completeness of recovery of gas released within the mouth of the greenfeed unit.

Sulphur hexafluoride.

A set of high flow SF_6 releasing permeation tubes were calibrated gravimetrically prior to use. Air contained in the aluminium canisters after removal from the back of the cattle (~2L) was removed from the canister using a diaphragm pump and transferred into a Tedlar bag. The concentration of SF_6 in the sample was then measured by gas chromatography (Hegarty et al., 2007). The concentration of methane in samples was measured in the Servomex analyser described for the respiration chambers. For this analysis, samples were diluted approximately 1 in 3 with nitrogen.

Feed Intake

Their inbuilt RFID recognition allows both the Ruddweigh and the GEM units to record how much ration and supplement (respectively) each animal consumed at each feeding event. The sum of all these values is currently being compared with the known weight of ration and weight of supplement placed in the feeder.

Faecal samples are currently being analysed for Cr and Yb concentration by ICP spectroscopy following digestion in perchloric acid. From these concentrations and from the known marker intake in supplement, faecal output will be calculated and multiplied by estimated digestibility to estimate feed intake. This will allow comparison of marker-based intake measures with the true feed intake measure. Knowing this will help us understand if we can determine intake and so methane yield, via the GEM unit by including a marker.

RESULTS

Diagnostics & evaluation of GreenFeed attributes

Air flow measurement

Comparison of air speed up the tower by the inbuilt sensor (located in the center of the pipe) with airflow measured by an inserted pitot tube showed a significant difference in air speed (Figure 5), and correction for sensor error was required after the experiment was completed.

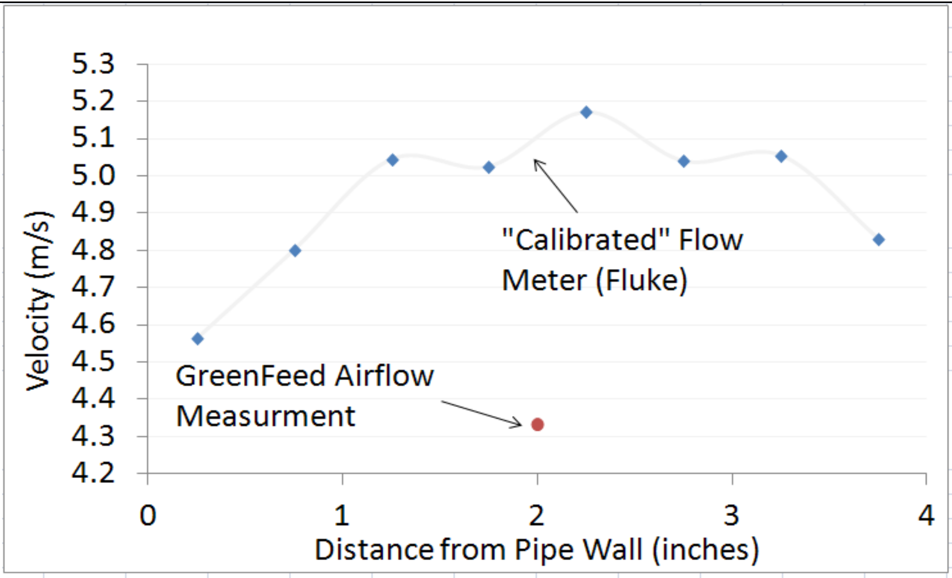
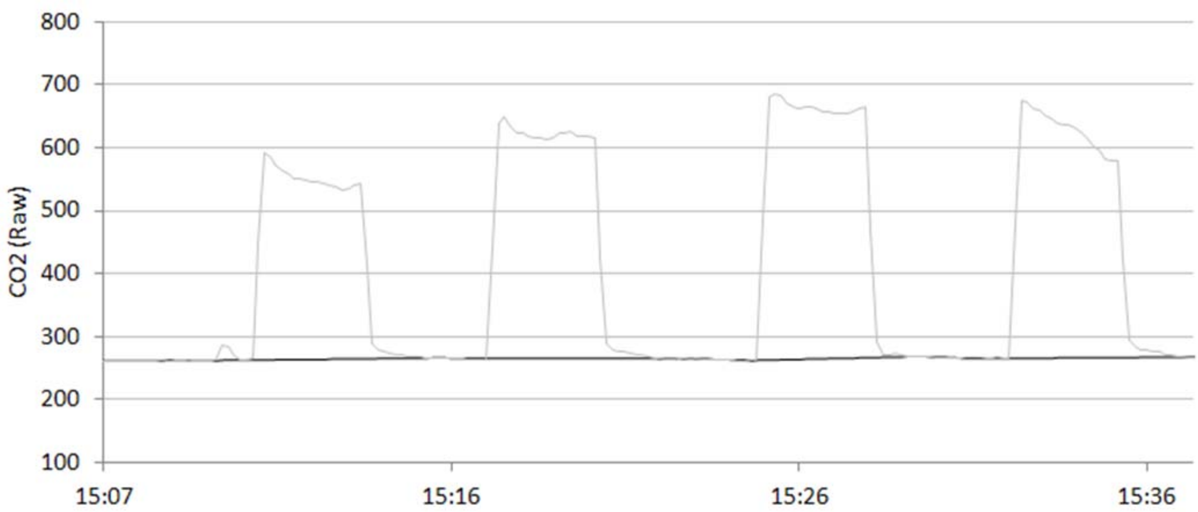


Figure 5. Velocity of air up the pipe as measured by the inbuilt sensor and a pitot tube used to calibrate the flow. Airflows were corrected for this discrepancy for all data presented

Recovery of added gas (CO_2)

To ensure that all gas exhaled or eructated by cattle was drawn into the GEM unit, small cylinders of compressed CO2 (paintball cylinders) were opened at the mouth of an operating GEM unit. The weight of decline of the cylinder was recorded over time to provide a measure of CO2 production rate. The GEM measure of emission was continuous over this period and the adjusted airflow (for reasons described above) was used to calculate CO2 flow rate by normal GEM procedures. Comparison of these gravimetric and flow based emission measures shows the GEM emission estimate to be within 2% of the gravimetric value (Figure 6a&b)



Test Number	Local Cellular Phone Start Time (nearest minute)	Mass of CO2 Released End - Start Weight (g)	GreenFeed Adj CO2 Mass (g)	Difference (%)
1	15:12	15.5	15.8	1.7%
2	15:18	19.2	19.5	1.3%
3	15:26	22	21.7	-1.3%
4	15:33	19.8	19.6	-0.9%

Figure 6. Increase in CO_2 concentration in air going through the GreenFeed system (A, graph) while 4 releases of CO_2 were made from cylinder gas and (B, table) the difference in estimated CO_2 flux between gravimetric and GEM flow based measures of CO_2.

This accuracy in emission estimation using the GEM flow system was true for both the supplement delivering and the water delivering GEM system (Figure 7).

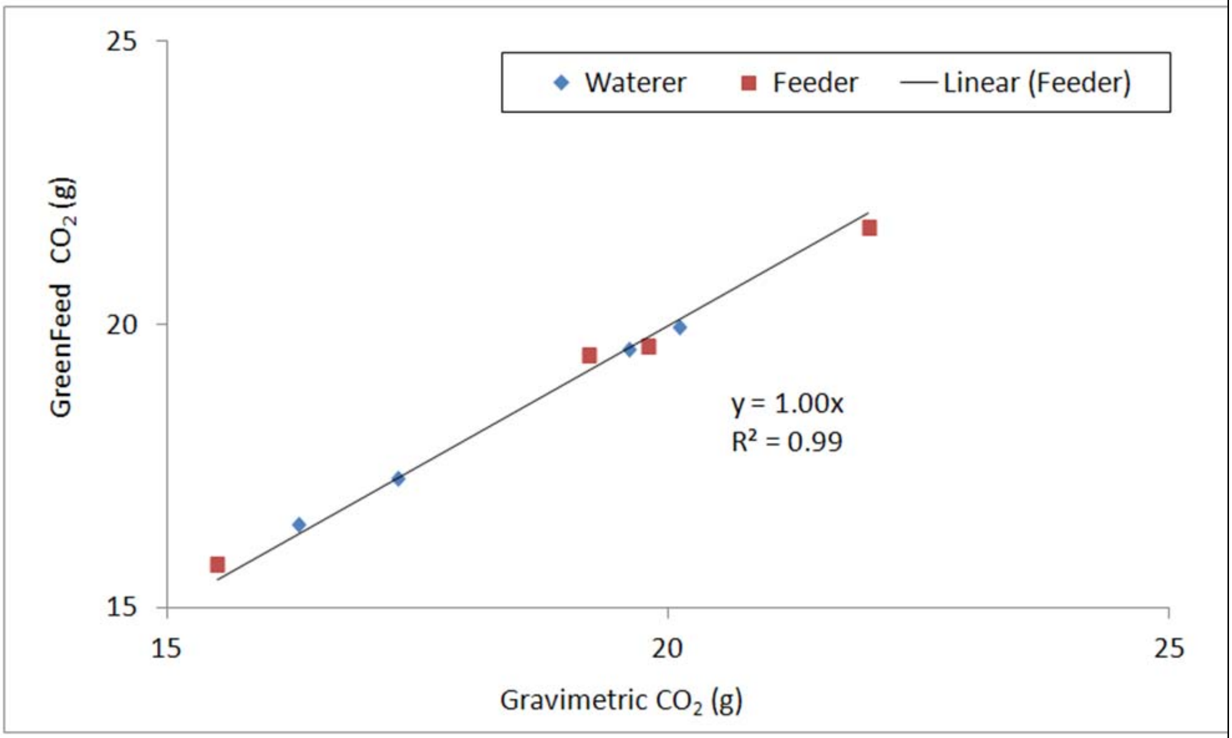


Figure 7. Comparability of CO_2 flux measured gravimetrically with that estimated by flow and methane concentration measures in the water-delivering and the supplement delivering GEM unit.

<u>Role of filtering of data in emission estimates.</u>

Unlike open circuit calorimetry where emission every second are counted or averaged; the GEM system does NOT simply average the methane concentration in the airstream and multiply by flow. There are two key aspects of including/excluding data from the emissions calculation. Firstly is that only emission occurring in eructation events are included. Typically there is a baseline emission (CH4 or CO2) and approximately every 40seconds, gas is eructated from the rumen and released into the feeding area of cows in the GEM eating from the GEM unit (Figure 8). Only blocks of 'spikes' (and these over 3-4 minutes) are considered for inclusion.

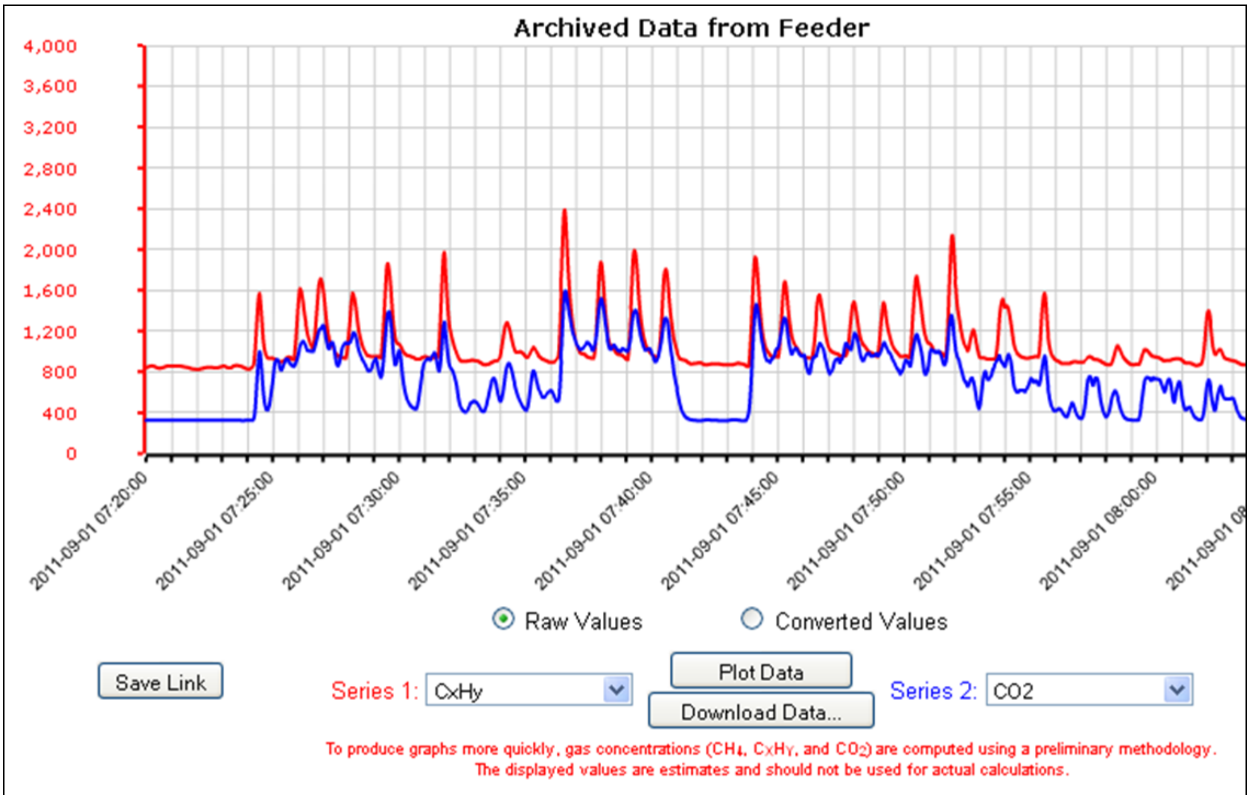


Figure 8: Screenshot of web interface showing typical plot of emission displaying the pulsatile nature of emission associated with eructation events (data are in arbitrary (signal strength) units – which GreenFeed staff call 'fudgets'). Methane has a very low baseline emission (thought to be lung sourced CH_4) and very sharp spikes of methane (thought to be eructation events)

Secondly, the 'head position sensor' in the feeding area identifies if an animal has its head near the air intake or is standing up and back where gas may be lost to outside the hood. An automated protocol (And we have not been privy to its algorithms) compares the methane or CO_2 spike occurrence with the head position sensor data. If the head is out of position then that portion of the emission spike that may be occurring at that time is not included in averaging of methane concentrations over that time window (Figure 9). This is a slight 'black box' screening procedure that the company applies to the data.

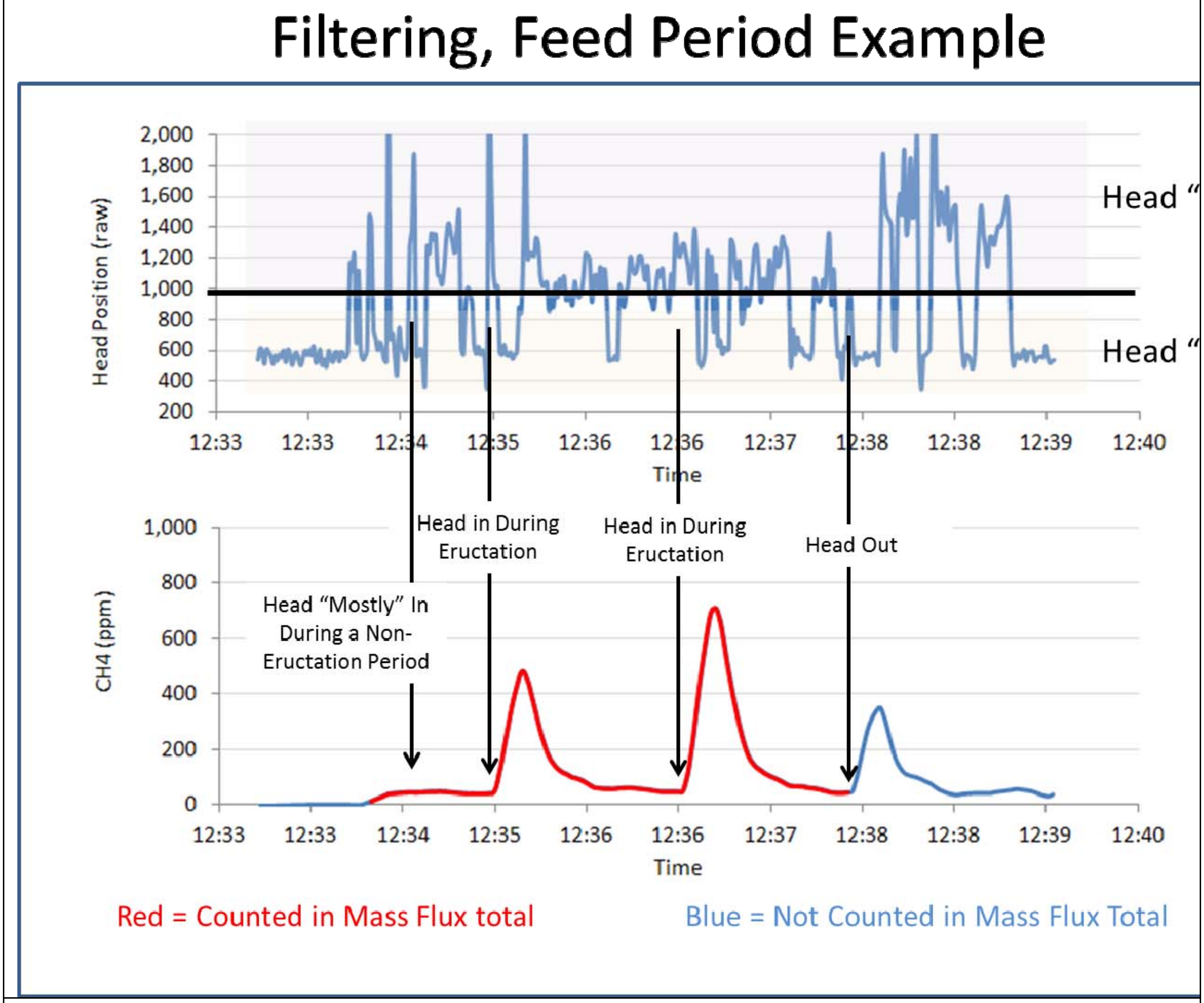


Figure 9. Alignment of eructation peak with head position over time, showing how only eructations occurring when the head is registering close proximity to the sensor are included in calculation of mean peak height from that batch of eructations.

Can Greenfeeds be used to partition ruminal from body CO_2 production ?

One of the novel things about the GEM unit is that it gives moment by moment gas concentrations, so that individual eructation events are observed. This cannot be achieved in respiration chambers where all air is mixed and typically retained in the chamber with a mean retention time of 5-7minutes. Consequently it is possible to separate out the CO_2 concentration in breath from that arising from rumen eructation. It is unlikely to be a perfect split, but may be useful and provide a mechanism to assess total rumen fermentation in the future. This is apparent in Figure 10. It is shown how the 'baseline' CO_2 can be averaged to get mammalian metabolic CO_2 production, and the peak CO_2 concentration averaged to get CO_2 production form the rumen. Indeed, C-Lock have built a GEM unit that can be programmed to direct eructation emissions down one tube and base respiration emission down another which would have great advantage for isotope studies.

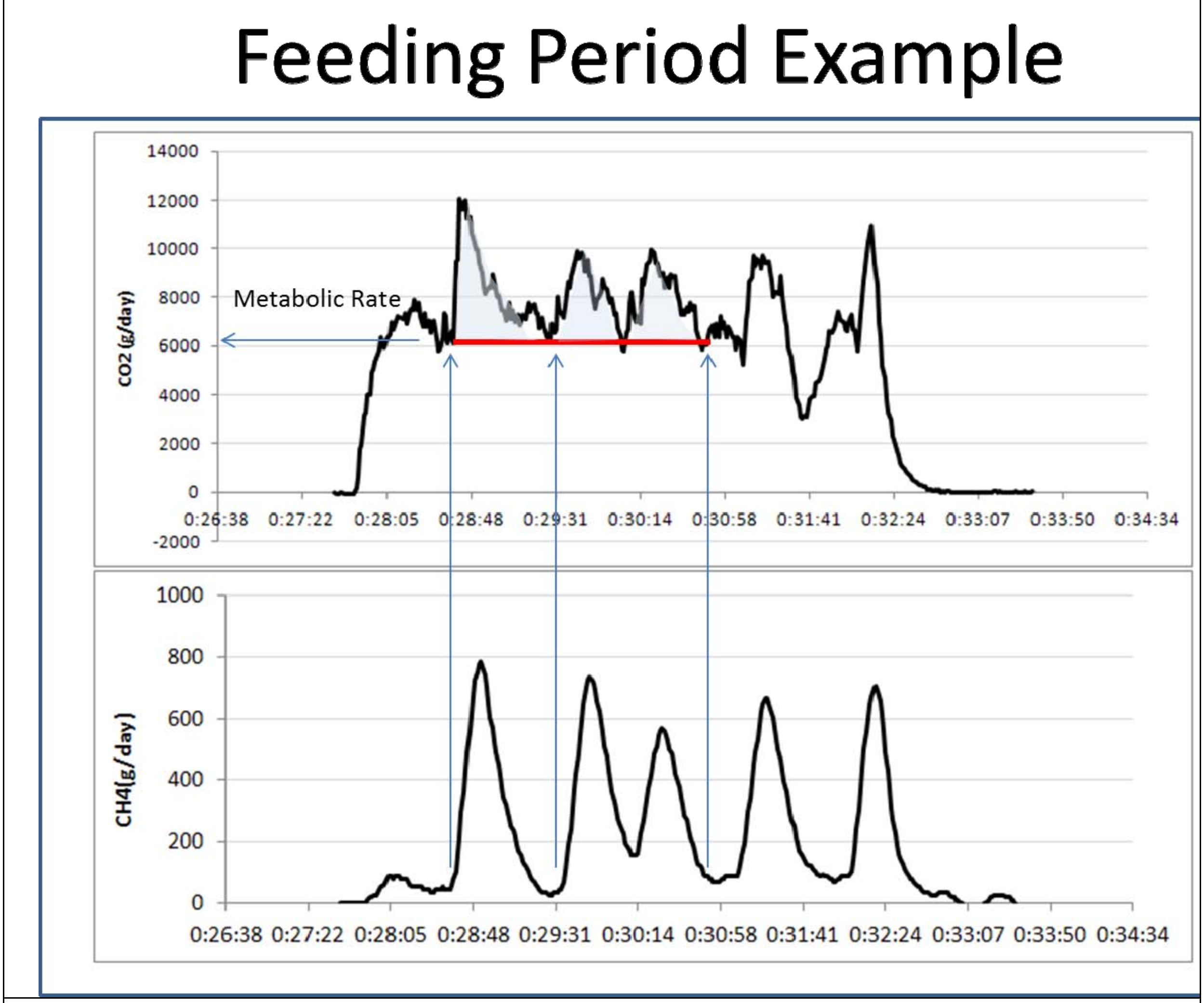
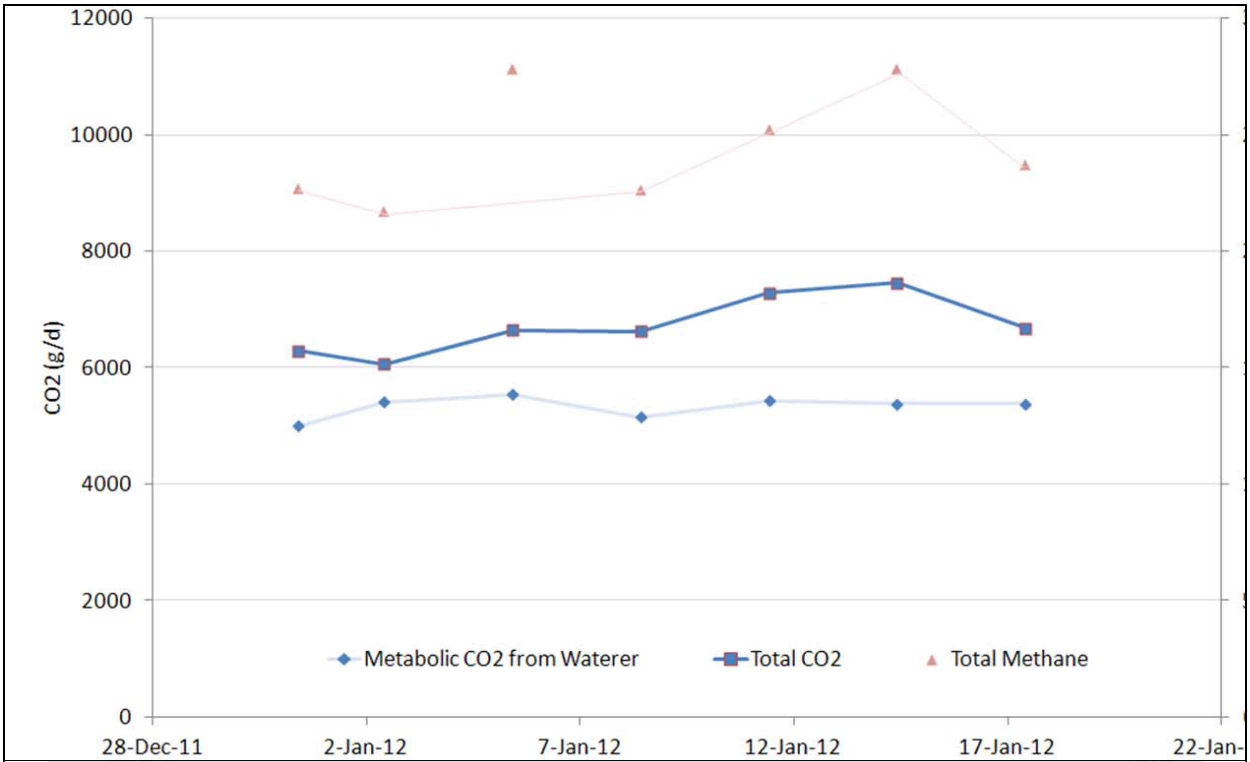


Figure 10. Demonstration of how the 'spikes' in CO_2 emissions that occur with eructations (apparent from contemporary methane emissions in lower graph), can be differentiated from the baseline CO_2 emission that probably reflect animal metabolic CO_2 in difference to rumen derived CO_2.

The data is supportive that such partitioning is appropriate. A study of the ruminal verses metabolic CO_2 production when so partitioned (Figure 11) shows that the metabolic CO_2 is quite stable over the day, whereas the ruminal CO_2 production follows a similar diurnal pattern to CH4 production



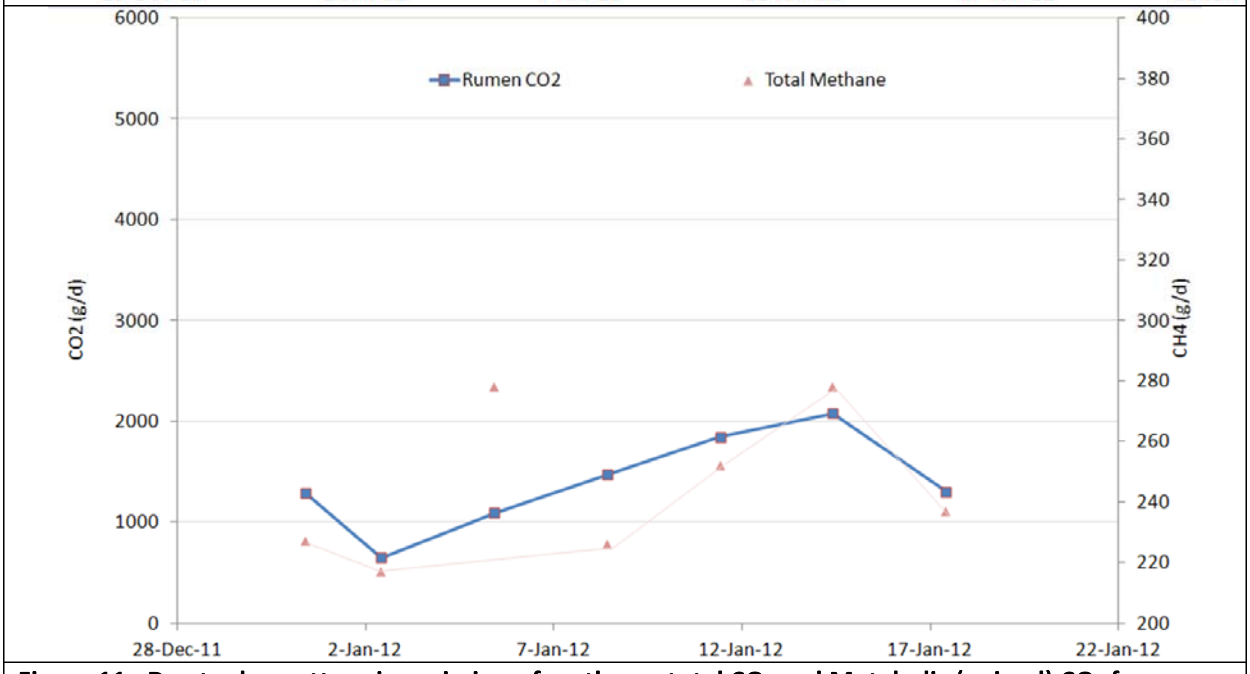


Figure 11. Day to day pattern in emission of methane, total CO_2 and Metabolic (animal) CO_2 from one heifer based on data from the water GEM unit (upper graph). On the lower graph the rumen CO_2 production (calculated as difference between total and metabolic CO_2) is shown and reflects a similar pattern to the methane emission, as may be expected if the two are stoichiometrically linked.

Repeatability of animal emissions

In this experiment, 2 of the 8 cattle were removed from the trial before measurement commenced. This was done because they did not become regular users of one or both of the GEM units in the 5 days of introduction. This was a harsh decision and in normal circumstances more time and strategies would have been applied to get them used to GEM units, but the experiment was tightly scheduled to allow access to respiration chambers.

The GEM units themselves showed a surprisingly high repeatability of emissions over time for ad-libitum fed cattle (Figure 11).

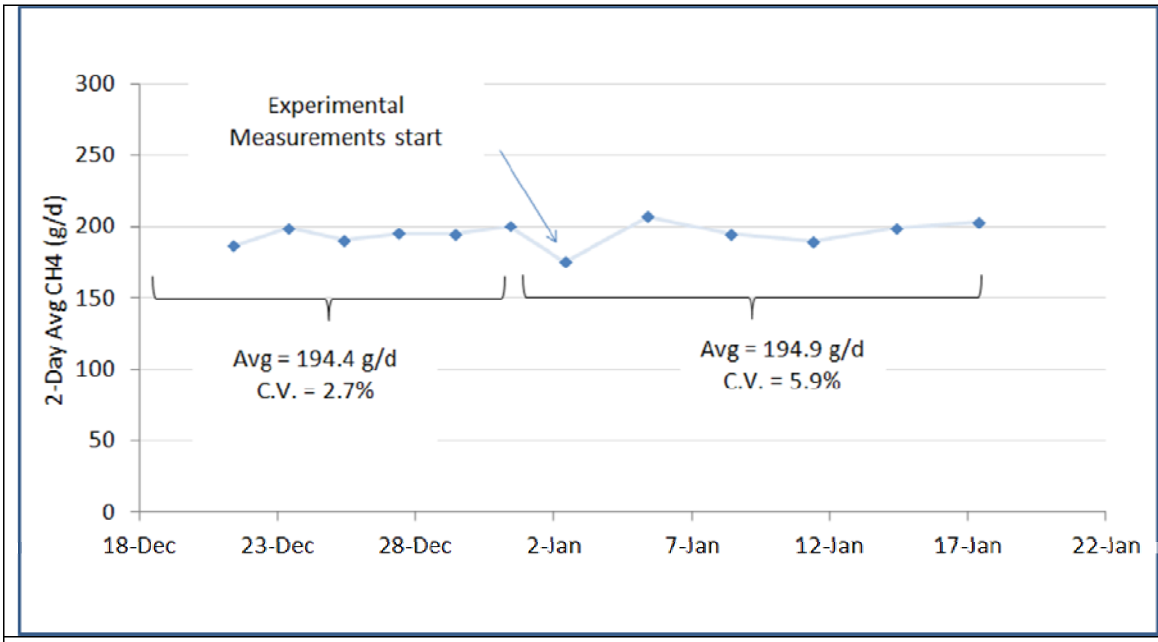


Figure 12. Sequential rolling 2-d average emissions based on 3 heifers (that ate similar amounts)

One of the challenges with the trial was that while in the pen, animals consumed feed *ad-libitum*, they often did not eat the same amount when in the respiration chambers. This made comparison of chamber and GEM emission measures difficult.

Study of the Methane Yields (g CH_4/kgDMI) showed the chambers to have the anticipated MY levels (Table below). For three of the cattle measured, MY was comparable for GEM supplement and Chamber emission measures. For animal 2066, intake was highly variable and it had the lowest overall intake, so it was hard to get confident emission estimates in this assessment where animals were in chambers for one day and on GEMs for 2. It is not clear why Animal 2417 showed marked difference in MY when measured by GEM or Chambers.

Table 1. Methane yield (g/kg DMI) of 5 cattle measured on 6 x 23h periods in respiration chambers, with each measurement being preceded by 2 days of ad-libitum intake and emission monitoring by a GEM unit. When in chambers cattle were offered the average voluntary intake of the preceding two days. Data for period 1 (P1) are shown in italics and were excluded from the average on the bottom line due to poor intakes the first time cattle entered the chambers.

CHAMBER DATA			Animal		
period	2019	3676	2293	2417	2066
P1	*22.3*	*20.9*	*14.3*	*20.3*	*wet feed*
P2	*21.8*	13.5	20.9	24.3	20.9
P3	16.3	19.4	23.3	19.3	19.7
P4	16.7	17.1	25.7	17.1	did not eat
P5	17.9	15.9	22.2	16.6	15.0
P6	16.0	21.5	17.6	16.7	23.2
Mean MY	**17.7**	**17.5**	**21.9**	**18.8**	**19.7**

Table 2. Methane yield (g/kgDMI) of cattle measured using the GEM supplement unit over 6 x 2d periods. All periods are included in the average as intake in period 1 was comparable to intake in other periods when in the open pen, as identified by the Ruddweigh feed intake recorder.

GREENFEED DATA					
			Animal		
period	2019	3676	2293	2417	2066
P1	*20.0*	*15.9*	*15.2*	*22.7*	*26.0*
P2	*16.9*	14.8	22.3	30.4	29.8
P3	16.0	16.8	22.9	22.9	25.3
P4	18.5		22.8	20.2	24.8
P5	18.5	23.6	20.2	23.0	19.2
P6	15.6	18.0	20.7	25.6	27.7
Mean	**17.6**	**17.8**	**20.7**	**24.1**	**25.5**

Average intake for individual cattle are shown below.

Animal	DMI (kg/d)	Methane production g/d
2019	12.715	222
2066	6.833	171
2293	8.578	174.8
2417	9.994	238.8
3676	12.488	226

<u>Water verses supplement GEM units</u>

The GEM unit that uses water as the bait rather than a pelleted supplement was constructed especially for this project so was a prototype. It has been anticipated that emission monitoring may be able to be made at the water source in feedlot or paddock situations, thereby avoiding the need for costly supplements and avoiding the supplement itself interfering with the intake of the basal diet and so natural methane emission levels. During drafting of this project, Growsafe from Canada were listed as participants but they proved reluctant to partner with either or both ourselves or GreenFeed for their own commercial reasons. Ironically, they wish to meet with us while the RELRP workshops are on in Sydney next week. Despite their lack of participation we learned a number of things about using the water based GEM that were different from the supplement system.

- More learning is required for cattle to use the water based feeder (they must activate the dispenser with their nose) & they were more reluctant start using this than a the supplement GEM unit. Since water is essential and more urgently required than feed, this requires careful monitoring.
- The total time taken in a drinking event is much shorter than for a supplement eating event. So the opportunities to capture eructation events is greatly reduced.
- The eructation pattern observed during feeding is NOT replicated while drinking. It is clear that while cattle can eat and eructate, drinking and eructating do not occur in like time patterns.
- The head position required for identifying a good series of eructations is quite different to the head position for good emission capture when eating.
- The data filter settings for gaining good emission data for the water GEM must be quite different to that for the supplement GEM unit. Both the time restrictions and the head position setting constraints must be changed. At this time these can only be changed by GreenFeed themselves, not by us as users.
- Because there are les eructation events around drinking, it is thought the water GEM unit may be a better approach to determining animal metabolic CO_2 production than is the use of a supplement GEM unit, should researchers want this measurement.

When the (signal) filters used for supplements were applied t the water GEM, emission measures were approximately 30% different between the 2 types of unit. When the filter settings were changed (by GreenFeed), this difference could be reduced to 1% (Figure 13).

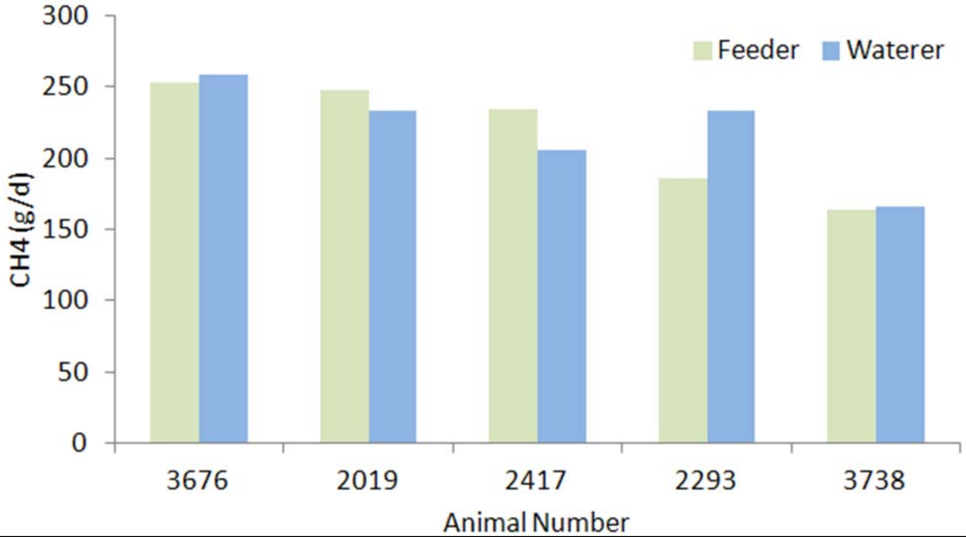


Figure 13. Average emissions for 5 cattle as determined by GEM units using supplement as the attractant ('Feeder') or water as an attractant ('Waterer').

Experiment 2.

Issues arising in Feedlot application of GEM supplement unit

As indicated above, it was thought that the water based GEM unit may be well suited to measuring emission in feedlots, where cattle are feed from open bunks or automated feed devices (such as those made by Growsafe), that do not allow emission measurement during feeding. However, there was not scope in the research year to get the aspects of water flow regulation optimised nor the signal filtering optimized. So this was not tested in the feedlot. Instead, the GEM unit was used and these findings have largely presented in Milestone 5 but are presented again below for completeness.

GEM units are expected to have application in emissions research for both grazing and feedlot cattle. The feedlot application is of immediate importance as major investment in emissions measurement for up to 5000 cattle is being sought in association with feed efficiency testing across the Beef Information Nucleus herds.

Key issues relating to measurement using short term measurements were identified through an experiment in which cattle were introduced to a feedlot in which the main ration was provided by an automated feeder and a GEM unit was located in the same pen.

Materials and Methodologies:

Design

20 yearling Angus steers (average LW of 320kg) were selected and allocated to two non-protein nitrogen treatments, T1 (n=10) and T2 (n=10), using stratified randomization. Rations fed were formulated to be isonitrogenous and isoenergetic.

Animal monitoring and treatments

Steers upon feedlot entry were administered vaccinations against the main 5 clostridial diseases (enterotoxaemia, tetanus, black leg, black disease, malignant oedema) using Pfizer Ultravac 5in1® and against *Mannheimia haemolytica* using Coopers Bovilis MH®. Steers received booster vaccinations of both vaccines 14 days post initial vaccination. Cattle were monitored daily throughout the course of the study for normal feedlot diseases and methaemoglobinaemia. Animals showing infections were administered long acting doses of broad spectrum antibiotics (penicillin) intramuscularly and closely monitored to determine if further intervention was required.

Diet Adaptation and Ration formulation

Upon feedlot entry, steers were acclimated to the two isonitrogenous and isoenergetic feedlot rations over a period of 29 days where inclusion rates of urea and calcium nitrate were gradually increased simultaneously with grain inclusion in the ration until final levels were reached. Details of ration acclimation schedules are provided in table 1 below and details of ration compositions are detailed in Appendix 1. At day 57, the finisher ration was altered in terms of urea inclusion rate due to a discrepancy in the nitrogen content of the two non-protein nitrogen sources which meant that the two rations were not exactly isonitrogenous initially. Steers were fed the final finisher rations *ad libitum* for a period of four weeks until the conclusion of the pilot study. Water was supplied *ad libitum* over the entire period of feeding. Steers were acclimated to the starter ration and intermediate 1 ration in bunk feeders and then fed intermediate 2 and the finisher rations in self-feeders where individual feed intake could be measured.

Table 1. Ration acclimation schedule of the treatment groups T1 and T2 from starter to finisher rations

Day	Ration type	T1 - Urea treatment (urea % inclusion, *as fed*)	T2 - Nitrate treatment (Bolifor % inclusion, *as fed*)
Day 1 – 7	Starter	0.25	1.00
Day 8 - 14	Intermediate 1	**0.60**	1.60
Day 15 - 21	Intermediate 2	**0.75**	2.10
Day 22 - 29	Finisher 1	**0.89**	2.57
Day 30-57	Finisher 2	**0.87**	2.57

Sampling and Data Recording

Animals were weighed weekly to determine individual and average liveweight gain (kg) and simultaneously bleed via tail venipuncture for methaemoglobin determination. Individual animal dry matter intake was recorded continuously over the duration of the study using self-feeders.

Feeds, feeding and feed analysis

Details of ration compositions are provided in appendix 1. Animals were fed *ad libitum* once daily at 0700hours throughout the study. Feed samples were taken daily and a pooled sample for each diet was analyzed for dry matter content, crude protein, neutral detergent fibre, acid detergent fibre, DMD, DMOD, inorganic ash, organic matter, metabolizable energy and crude fat.

Methane Measurement

Because only 1 Greenfeed unit was available it was necessary to swap the unit between the nitrate and urea groups. This change was made at weekly intervals. The (6mm diameter) pellet delivered through the GEM unit was a commercial lucerne-based horse pellet, and cattle had no prior experience or familiarity with this pellet.

KEY ISSUES:

1. Communications capacity
    - Since delivery in December the wireless modem (with SIM card providing internet connectivity) in the GEM unit had not worked, despite 2 replacements being tried as provided by GreenFeed. While only a 'teething trouble' it has taken an inordinate amount of time. The fact that such things as replacing modems (or many other pieces!) depends on GF intervention either from USA or via software that they send for the purpose means getting maintaining communication has been a big impost.
    - In principle, data is able to be collected while the machine is offline (no internet connectivity for some reason), with the data being automatically stored on an installed USB thumb-drive. However, sending this data to the US 'after the event' is like eating one pea per mouthful.. it takes a long time to get the data through. The collected data has to be split up into multiple files and loaded on-line subsequently as multiple files. GF have provided software to do this but it is a most inconvenient and undesirable process and DOES NOT ENCOURAGE USE OF GEM UNITS WHERE CONTINUOUS INTERNET ACCESS IS NOT AVAILABLE.
    - Tullimba feedlot is some 60km from Armidale and is in a poor mobile-phone reception area, with typical mobile-phone connections dropping in and out subject to the exact location of the user & type of phone. However, once the modem was operational, there has been no trouble maintaining GEM connectivity to the internet from this site. This is because the GEM unit has an aerial mounted along the side of the flow tower.

2. Maximising animal access to the GEM unit.
    - In the previous experiment, 2 cattle (of 8) had to be removed from the trial because they did not adapt to the GEM water dispenser or supplement dispenser quickly enough to allow their inclusion (5d).
    - In this study, the GEM unit was installed at the end of a 2.5m race-way, which is required to restrict access to one animal at a time and allow correct RFID identification (Figure 1). The fan is turned off in these early days to avoid fan-noise scaring cattle away, and to ensure that the smells from the feed come out of the unit and are not sucked immediately into the exhaust system. The dispensing system was set so there was no limit to the number of times supplement could be dispensed to any animal (normally this is set at about 5 feeds/d) However, entering a narrow race when there is no knowledge of a reward proved not appealing to cattle. Over the first week of GEM access in this environment, it was estimated that only 3 of the 10 cattle accessed the supplement (and no emission measures were collected as the fan is turned off so there is no flow past the sensors). The others all ate the main ration from the automatic feeder but did not walk down the GEM race and activate the pellet dispensing process.

Figure 14. GEM unit installed at the end of a 2.5m race within a feedlot pen. Cattle did not learn to access the feeder readily (3/10 after 7 days).

- In the 2nd week, the race was removed and transferred to the 2nd group so that steers could access the GEM feed tray together without restriction (Figure 2). This proved far more successful with cattle jostling for access. Importantly, the software can be made to release feed with no regard for a unique RFID eartag being present.

Figure 15. GEM unit installed 'flush' with fenceline so that cattle were not required to enter a raceway before accessing the unit.

- The inbuilt bell that sounds when feed is dropped is showing itself very effective in attracting animals, with animals coming from the far end of the pen when it rings during set up of the unit.

3. Animal Memory
    - Once adapted to the unit and removal of the unit for 1 week (only period tested) does not affect the willingness of cattle to enter. They do not appear to have to 'relearn' what the feeder provides or have any neophobia abut it returning to their pen. They go back to it immediately, meaning we can expect to be able to use one feeder to service more than one group of animals over time by rotating the location of the unit between pens and groups.

4. Unit Stability

There are multiple components to calibrate in the GEM unit. These include:

- The air flow profile across the diameter of the exhaust tower. This is an irregular calibration, perhaps every month & is used to convert data on flow rate measured mid pipe (as continuously monitored by a flow sensor) to total flow up the pipe. Recovery tests with CO_2 showed that the FLUKE flow meter provided to check this flow was malfunctioning. It has been sent back to the USA but has not been repaired and returned to us.
- Calibration (spanning) of the Methane, CO_2 and hydrocarbon sensors. This is done every 3-7d but we get fairly regular calls from GF asking us to recalibrate on additional occasions. If further GEM research is funded we will seek to get this calibration automated.
- Calibration (Baseline setting) of the methane sensor. AN unexpected need was a request to rescale the methane sensor because the electronic drift over several months (when the unit has mostly not been in use), was too great to give a useful working range. This baseline reset involved dedicated software & direct connection to the sensor under guidance from GF.
- Recovery test (internal). The GEM unit has an internal canister of pressurised propane. At programmable intervals, the unit will automatically release propane into the feeding area from where it is drawn through the system and the size of that propane peak is monitored on each occasion. If the peak area changes, either a sensor or airflow problem is being experienced and an alarm is sent off in USA generating a quick email to tell us to undertake further checks.
- Recovery (external). A gravimetric test is conducted in which miniature CO_2 (paintball) canisters are opened in the face of the unit and the decrease in weight over time monitored and compared with the CO_2 entry rate estimated from the GEM unit by multiplying flow rate x CO_2 concentration. This is an infrequent test (expect every 6 months).

CONCLUSIONS

The GreenFeed emissions monitoring units can provide accurate and stable estimates of daily methane emissions from cattle that are comparable to measures from open circuit respiration chambers. GEM units also determine daily carbon dioxide flux from cattle, and therefore have a role in quantifying animal energetics and energetic efficiency. It is likely that the data can also be dissected to quantify ruminal and animal metabolic CO_2 production differentially. Since it operates on 12Volts (so can run on solar cells), it creates a large number of possibilities for emissions research/verification and for feed efficiency research in animals grazing pasture. Two key attributes that we have not got to define in this period are the number of animals that can be effectively serviced by a unit and the long term functionality in a paddock setting. The water unit has particular advantages (such as potentially not needing topping up daily, not introducing 'unnatural' energy substrates into the diet and being an essential nutrient-so there can be no shy feeders and all animals will be recorded). However, being a first construction prototype, we found practical constraints that will require further attention. In particular the water flow meter used, the pressures involved and the head-position and time filters that are applied in data processing.

Both units needed very frequent attention to calibrations or testing of some sort and there are multiple sensors that need calibration or testing at a range of intervals. We believe for truly remote rangeland use, an autocalibration routine for CO_2 as well as methane will be required, as it has for propane.

We have identified the importance of providing open access to the unit with no fans, raceways or feed constraints in the introductory period and we have discovered that we are able to remove the unit form animals for a week and they immediately recommence use of the unit when it is returned.

We found support from the manufacturer immediate and there has been near daily contact during campaigns, and trouble shooting, part replacement etc in between. Their advice was not always right, was sometimes overbearing but we believe was consistent with being smart scientists wanting to respond immediately to any difficulties with the operation their device. While we have not yet achieved it, we understand we will be able to operate the instrument without reference to C-Lock in the future, although data will still be processed through the web interface and emailed to us automatically.

In conclusion, GEM devices are very smartly designed as an analytical tool. Their manufactures are not animal scientists so there are some aspects of suitability for field use that can be improved. However, the complexity of internal operation, of algorithms and calibration and internal checks is very impressive. While the devices are expensive and is in some aspects patent-protected, it would seem appropriate to assist in further development of this tool and inappropriate to try and 'reinvent the wheel' by replicating another stand-alone device for similar purposes, even if no patents prevented it.

In summary, it has largely been a full time job keeping the unit running. The expectation is that most of these 'hiccups' will stop occurring as they are almost all electrical in nature, yet it has been several months of near-daily communications with C-Lock to overcome each new problem. To their credit, C-Lock have identified these problems as they see them in incoming data and have provided the parts, software, advice (or all 3) on how to repair them, usually at their expense. There has been some concern here that C-Lock have on occasion provided the wrong component or software, wasting staff time here. Also, data is slow to return from the USA (rarely overnight delivery) & this makes monitoring of emission levels difficult.

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