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Evaluation and optimization of greenfeed emission monitoring units for livestock

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Executive summary

GreenFeed Emission Monitoring (GEM) Units are a commercially developed measurement system that allows estimation of daily enteric methane production (DMP) from cattle based on averaging multiple short term (3-6min) measures of methane emission. GEMs were developed in 2010 but had been little tested. This project was responsible for validating the accuracy of DMP estimation by GEM, for developing guidelines on the number of samples that are required to establish emission reduction and the emission phenotype of an individual. In addition the project led to the development of a commercial sheep GEM unit. No differences between GEM and respiration chamber determined DMP were detected, so confirming the accuracy of the units, with units being used to demonstrate effects of nitrate and of animal genotype on DMP in research trials and demonstration sites. The GEM unit has commercial application in (1) phenotyping cattle (and potentially sheep) for their methane phenotype as part of a genetic improvement program; (2) verifying on-farm mitigation and (3) underpinning inventory estimate estimates is used in the field for prolonged periods. There is need for evaluation of potential GEM impacts on grazing behaviour and emissions in more extensive environments to provide assurance that the process of being measured does not interfere with the natural behaviour and emission of the animals studied.

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1. Background

The majority of understanding of animal energetics and daily methane production (DMP) has been obtained from indirect calorimetry using open or closed circuit respiration chambers (RC) (Blaxter 1962, Blaxter and Clapperton 1965). Increasingly however there is a demand to measure ruminant methane emissions in animals within their natural production environment. This demand arises from the need to verify inventories, verify mitigation claims on-farm and measure large numbers of animals to determine the genetic parameters of methane output (McEwan *et al.,* 2012). Practicality prohibits use of RC on-farm and their use for hundreds or thousands of animals is occurring but is very expensive (Arthur *et al.,* 2012). Robinson *et al.,* (2010) identified short term measures of methane production were strongly correlated with DMP and can be used to obtain short term on-farm emission data without need for a RC.

An overview of direct short-term measures of methane flux

While respiration chambers are not used for determining DMP on-farm, two other approaches do allow long-term measurements of DMP of grazing animals on-farm over 2-15d. These include the polytunnel system (Lockyer and Jarvis, 1997; Murray *et al*., 1999) and the SF_6 tracer method (Johnson *et al*., 1994). The polytunnel system acts as a multi-animal respiration chamber with no floor, with an inflatable tent placed over a small pen of animals on pasture. It is reliant on a large exhaust fan drawing air in through the unsealed edges of the tent (eg. where the tent meets the ground and where door-flaps meet), so that all emissions exit via the exhaust tunnel and fan where gas concentrations and air flow are measured. This system allows animals to demonstrate diurnal grazing pattern and diet choice within the confined area. Because emissions can be measured continuously for extended periods, it is not considered a short term emission measurement and is not discussed further. The SF_6 method also samples expired gases continuously over multiple days while allowing the animal unlimited access to its grazing environment, so is not a short term emission measurement. The SF_6 method has been compared with other RC measurements in a number of studies and will not be considered here (eg. Grainger, 2007; Pinares-Patiño *et al.,* 2011).

GreenFeed Emission Monitoring Unit

The GreenFeed emission monitoring unit (GEM; Zimmerman, 2013) is a patented device manufactured by C-lock Inc. (Rapid City, South Dakota, USA) which measures emissions from individual cattle repeatedly over 3-6 min periods whenever they visit the unit to consume a delivered supplement. Air is continuously drawn into the shroud where cattle are supplemented and methane (and CO_2) flux are calculated continuously by multiplying the methane or CO_2 concentration by the flow rate of air exiting the shroud. Completeness of trapping of gases released in the shroud is tested manually by determining the change in CO_2 flux associated with release of a known (gravimetric) release of CO_2 into the shroud. Propane gas is released automatically at irregular intervals (when no animals are present at the unit) to check for sensor drift of the hydrocarbon sensor, and gas sensor units are manually calibrated at approximately weekly intervals. During testing, the units have been primarily used with cattle in dairy and beef research facilities offering high feed availability, but they could be used in remote rangeland locations. The unit does not determine emissions lost in flatus. The GreenFeed unit is designed to be accessible to cattle at all times, so the prospect for emission measures being made at all times of day or night exists and this could minimise the risk of biasing estimation of daily flux by sampling at only one stage of the feeding cycle.

At the time of study commencement, there was no data from the manufacturer or independent sources comparing GEM estimates of daily methane production (DMP; g CH_4/d) with measures made in respiration chamber. Consequently there was a need to verify the DMP estimates as well as evaluate the robustness, and practicality of the GEM system and make it applicable to sheep.

Contractually, this project was funded on the basis of *" a growing requirement to prove mitigation is being achieved on-farm as scientists investigate a wide range of livestock production practices; develop new means by which landholders can reduce methane emission and seek to convert current technologies into practical solutions for landholders"* In seeking to meet this need the project team committed to the following outcomes:

- A GEM cattle unit capable of operation in a remote location for at least 7days without human intervention.
- Optimise the construction and operation of GEM units for long term use in grazing environments
- GEM units for sheep available and accuracy verified.
- Standard international protocol drafted for use of GEM units to verify emissions mitigation in sheep and cattle.
- Published confirmation of whether the measurement process itself (using GEM units) causes changes in animal grazing behavior.
- Peer reviewed publication documenting correlations between GEM and respiration chamber emission data.
- Validation of estimates of daily methane Production from cattle GEM unit optimised

2. Methodology

All the research undertaken has either been written up and submitted for publication, or is drafted into manuscripts that are well developed towards publication, so the methodologies are only reported in overview in this report which is primarily identifying the value of the work undertaken.

2.1 Validating GEM estimates of Daily Methane Production against respiration chambers

The process of comparing two methods of measurement is not simple, depending not just on the precision of the 2 methods, but the principles on which they operate. GEM units are made to be employed so that animals may access the unit and have emissions measured 3-5 times/d by computer regulation of supplement dispensing. As a result, it was expected that scheduling supplement delivery to incorporate any diurnal cycle in emissions would be important. There is also likely to be no regulation of intake of the basal feed, and since feed intake is highly correlated with DMP, there is a need to know and allow for the intake occurring during GEM measurement when making the respiration chamber (RC) study with which to compare emissions. In the practical environment many cattle will access the one GEM unit so it is important that cattle have access to the GEM in like manner to in the production environment.

After consultation with other GEM users, C-Lock and a biometrician, we developed a protocol based on multiple cattle having their voluntary basal feed intake (by auto-feeder) and DMP (by GEM) monitored for several days (2-4), then animals being fed the observed average *ad-lib* intake while in a RC for 24h. Animals were then removed and the process repeated with up to 6 periods of paired GEM:RC measurement per animal. This was developed in difference to other approaches where people had given cattle only limited access to GEM after feeding (thereby biasing the GEM DMP estimate), or where people fed the entire daily ration through the GEM, which is not realistically reflecting how cattle will access them in the paddock or feedlot, and likely to overestimate the alignment between GEM and RC estimates of DMP.

Two experiments were conducted to compare Daily Methane Production (DMP gCH_4/d) as estimated by GEM with emissions measured in RCs. The papers have been submitted and revised but are not yet published so methods are reported below.

In Expt. 1, cattle had 18 d to adapt to the diet, environment and GEMs but had no RC experience prior to the study. One animal exhibited high feed refusals in the RC so all data from this animal was excluded from analysis. Five female Shorthorn cattle of varying age and varying in live weight (LW) from 392 to 680 kg (518 ± 132 kg LW) were group-housed in a pen (8 m x 12 m) in an open barn with access to an outside exercise pen (10 m x 12 m; Figure D1). The range in animal LW was chosen to induce variable voluntary feed intakes so the emissions would span a range of DMP. No cattle were lactating but one animal was in the first trimester of pregnancy. The main ration was a lucerne/oaten chaff blend (Manuka Stockfeeds NSW; 8.8 MJ ME/kgDM; 11.2%CP; 55% NDF, 33% ADF). The chaff was provided *ad-libitum* to the group through a “Ruddweigh” feed dispenser with cattle identified by radio frequency ear-tag

(**RFID**), so that each meal of each individual animal was recorded (Bindon, 2001). The weight of all meals consumed in a day was summed and compared to the known weight of chaff added to the feeder daily to confirm the accuracy of the scales in the feed dispenser. In addition to the chaff, cattle were also provided with a measured quantity of pelleted supplement each time they accessed the GEM unit (13.2 MJ ME/kg DM, 12.7 %CP, 24% NDF, 11% ADF, 3.1% fat). When in the RC each animal was offered a quantity of mixed chaff and of pellets equal to its voluntary consumption of these feeds (from Ruddweigh and GEM units) over the 2 days preceding chamber entry. As it is known that methane production is affected by intake not only on the day of measurement, but at least on the preceding 2 days (Robinson *et al*., 2011), the DMI used in calculation of CH_4/kg DMI by all techniques was the mean intake (chaff + pellets) on the day of measurement and of the 2 preceding days (i.e. for RC = chamber day + 2 preceding days in pen; for GEM = intake in previous RC day + intake during two days in pen).

The conduct of Expt. 2 was as for Expt.1 except that ten Aberdeen Angus steers (365.2 ± 50 kg LW) were used, commencing after steers had been adapted to the diet, GEM unit and facilities for 21 days including multiple training periods in RCs. There were no animals removed in Expt. 2. In difference to Expt. 1, there were slight diet differences with the chaff (ME = 9.4 MJ ME/kg DM; 13% CP) and GEM supplement (ME=9.5MJ ME/kg DM, 13.4%CP, 25% NDF, 7% ADF, 2.7% fat) and feed allowance in the RC was based on intake averaged over 3 d prior to chamber entry.

Respiration Chambers

Five open circuit respiration chambers were used in Expt. 1 and ten chambers used in Expt. 2. Chambers (3.6m x 2.4m x 2.4m) were constructed of polycarbonate sheet (4mm thickness) fixed to a hot-dipped galvanized frame (Hegarty *et al.,* 2012). The chambers did not have a floor but were able to seal into a water-filled rebate in the concrete floor and then be lifted by pneumatic rams to allow cleaning. Within the polycarbonate box, a pen made of steel cattle panels (3m x 1.8m x 1.8m) was bolted to the floor to confine the cattle and avoid them causing damage to the surrounding polycarbonate chamber. Cattle entered and exited the RC by a polycarbonate door fitted into the chamber frame and a steel gate on the internal pen. The daily feed allocation (chaff + supplement) pellets was provided as a single meal immediately prior to sealing the chamber. An air flow system (outside shed – chamber – flow meter – high pressure fan) drew fresh air through each chamber (approximately 1400 L/min), with the rate of flow though the exhaust line from each chamber measured by an SCI mass flow meter (Model ST75V, Fluid Components International, San Marcos, California USA). A subsample of air from each chamber (2L/min) was continuously drawn from the exhaust line adjacent to the site of the flow meter, dried using a refrigerated drier (4^{o}C) and passed through a multiplexer. The CO_2 and methane concentrations in these dried samples of exhaust air from each chamber and a dried sample of concurrent ambient air were determined consecutively throughout the 24h measurement period, with the dried sample pumped into a Servomex 4100 analyser fitted with GFx infrared sensors. Each sample took 60s for purge and analysis so each chamber was measured every 12 minutes. The sample drying, analytical and data processing software were configured by AZCO Holdings (Auckland NZ). The gas analyzer was calibrated each morning using a standard mixed gas and recovery of methane through chambers was checked by introducing a standard pulse of methane (99% purity) before and after each experiment, with all emission data corrected to 100% methane recovery. Animals were randomly rotated though a chamber, ensuring each animal was measured in a different chamber in each period.

Predicted methane yield

MY and DMP were also predicted from the gross energy of the feed and the Intergovernmental Panel on Climate Change (**IPCC**) emission factor (IPCC, 2006) for comparison to the experimental measures. In the present study, gross energy intake (**GEI**) was calculated from the chemical composition of the feed assuming a 19% loss of the apparently digestible energy (excreted in the urine and as methane; McDonald *et al.,* 2011). Metabolisable energy (**ME**, MJ/kg DM) was calculated using the prediction equations recommended by the Australian Fodder Industry Association (2011) laboratory methods manual for roughages other than silages (ME = 0.203 * DOMD (%) – 3.001). Digestibility of the organic matter in the dry matter (**DOMD**) was estimated using the Pepsin-Cellulase method (Australian Fodder Industry Association, 2011).

Figure D1. Shorthorn heifer in Experiment 1 accessing GEM in group pen prior to entry into respiration chamber for comparative emission measure. Cow behind is consuming chaff from an automated feeder which records to quantity of chaff consumed.

Mixed model analyses were conducted for CH_4/kgDMI and CH_4/d (covariate-adjusted for DMI) in Genstat (Payne *et al*., 2011), using the residual maximum likelihood (REML) procedure. Animal was fitted as a random effect, and measurement technique as a fixed effect (RC, GEM-supplement, GEM-water when present). This simplified model was adopted after first testing for day and period effects. Including 'day' (of measurement) as a covariate showed no effect for DMP or MY (P=0.4–0.8), justifying the assumption (of no time-trend) which is necessary for the analysis of this systematic random design. Residual plots were used to check the validity of the underlying statistical assumptions of homogeneity of variances and normality. The estimated means for the measurement techniques were subjected to protected least significance difference testing (at the 5% level) in Expt. 1. With the prolonged multi-period design, there was the possibility of animals adapting differentially to each measurement technique over time (e.g. as they became more familiar with the GEM unit and with confinement in RC units). This was investigated in both experiments by fitting the period x technique interaction (for CH_4/d and CH_4/kg DMI). 95% confidence intervals were calculated for the measurement techniques in each experiment, and used in the combined analysis of both experiments. RC and GEM-supplement means from both experiments were pooled and a statistical hypothesis test was performed. All 95% confidence intervals were compared against the IPCC predicted emissions. Steers in Expt. 2 were ranked from the lowest and highest according to their averaged DMP and correlation between methods was calculated (using the Pearson coefficient). In Expt. 2 diurnal variation in DMP estimates was investigated using a spline model which included animal as a fixed effect.

2.2 Development and validation of sheep GEM units

Sheep GEM units had not been designed nor constructed prior to this project. Email interactions were held between C-Lock, UNE and DairyNZ (Garry Waghorn) early in the program to identify the key attributes required in the system and these were assimilated by C-lock in designing the unit. The unit was delayed while a new wide-ranging methane sensor was developed for C-Lock to be used in all sheep and future cattle units.

The units (3) were ultimately built, with 2 being sent to UNE and one to New Zealand. Dr Arjen Jonkers (AgResearch) visited UNE to assist in the validation study and UNE staff will travel to UNE before July 30 to help install and operate the recently shipped NZ sheep unit.

Two validation studies were undertaken with the sheep GEMs in like manner to the cattle GEM validations described above. In both studies sheep were group-house and had *ad-libitum* access to chaff provide through an automated recording feeder (local manufacture) as well as having access to the sheep GEM unit able to dispense up to 5 supplement deliveries/sheep per day.

Experiment 1

In study 1, eight sheep (48.1kg +_3.18) were group penned in an open pen (21m^2) within a shed fitted with a raised slatted floor. Each pen was serviced by an auto-recording feed dispenser and a sheep GEM, with water continuously available.

The basal ration was a commercially prepared blend of oaten and lucerne chaff (approximate ME 9.8, CP 15.3) made available to all sheep in a pen. Sheep were accustomed to the chaff for 30d before the study and to accessing the chaff from within the feeders for 15d. The feeders did not restrict feed consumption as a hopper containing 16 kg of supplement pellets was refilled regularly throughout the study. The RFID eartag of a sheep was recorded as it placed its head into the feed bay and the weight of feed on sheep entry and at the time the sheep removed its head from the bay was recorded, allowing the quantity of feed consumed per meal to be determined. A short panelled race (0.9 m) was installed in front of the feeders to prevent more than one sheep accessing a feeder at one time (Figure D2).

The SGEM unit was designed and constructed by C-Lock Inc. (Sth. Dakota, USA). The principle of operation was the same as that of the cattle GEM unit (Zimmeman 2013), however the design was scaled down in accordance with the live-weight of the sheep (Figure D3.). The air flow thought the shroud was 14.3L/s the sensor unit was different, and the quantity of supplement delivered per delivery was 8g/drop. The supplement dispensed by the SGEM was a locally produced cereal based pellet (Prydes EasiRide Pellets), During the study, the SGEM delivered 4 drops/meal with a minimum of 2h between each meal.

Figure D2. Auto-recording feed dispenser providing *ad-libitum* chaff to 8 sheep/group

Figure D3. Sheep GreenFeed Emission Monitoring unit used to monitor emission for 2d-day periods prior to sheep emission being measured by respiration chambers

The study was conducted over 4 periods. Each period was of 3 d duration with day 1 and 2 being in the group pen while their ad-libitum intake was determined and their daily methane emission recorded by the SGEM unit. On the third day, all sheep were transferred to respiration chambers (n=8) and offered a quantity of feed (and also pellets) equal to the individual animal's mean feed intake observed over days 1 and 2. Feed was provided to animals as a single allocation on entry to the chambers and sheep were held in chambers for 22h before being returned to the open pens for another period of SGEM monitoring.

Recovery of gases through the respiration chambers was tested in the month prior to commencement of this study and the recovery across the 8 chambers ranged from 98-103% of methane infused from a

cylinder fitted with a mass flow controller. Recovery of CO_2 was also >99.5% through the sheep GEM based on a 20 minute continuous release.

Experiment 2
The second study was conducted in the same locations as experiment 1, commencing several weeks after study 1 but with different sheep (n=8). Feed was an oaten chaff/Lucerne chaff blend as previously described, and was also delivered by the shared feed dispenser with automatic intake recording. Four measurement periods were used for all animals, with days 1 and 2 having feed provided by the auto-feeder and emission measured by GEM, while on the third day sheep were placed in individual respiration chambers (n=8) and offered their average voluntary intake of the preceding 2 days.

Statistical methods
GenStat was used to assess measurement method as a fixed effect. Initial analyses looking at sheep and period as random effects and DMP was adjusted by use of DMI as a covariate, where DMI was the average DMI for the day of measurement and the preceding day. REML analyses were then run in individual and combined experiments with sheep, periods and experiment as random factors.

2.3 Application of GEM units in on-farm demonstration and measurement

A number of AoTG and NLMP opportunities have arisen to apply the cattle GEM units in field campaigns to provide their robustness and utility. These opportunities include:

2.3.1 Measurement of 60 cattle of divergent generic merit for RFI while grazing (at Glen Innes NSW) and while finishing in a feedlot (Tullimba NSW)

An intensive study was made of the emission and intake of 60 genotyped Angus cattle of divergent genetic merit for residual feed efficiency (RFI) was made as described below.

The cattle were tested for weight gain and methane and carbon dioxide production. The weaned cattle were transferred to NSW DPI Agricultural Research and Advisory Station, Glen Innes, where all animals were managed together on native pastures to reach approximately 300 kg LW by 6 months post-weaning. During the test period the steers and heifers grazed separately in adjacent paddocks of improved pasture of approximately 14 ha each and all animals had unlimited access to reticulated water. Each paddock contained a single Greenfeed emission monitor (Fig. D5, and described below) and the cattle had continuous access to a GEM throughout the 42d grazing experiment. The cattle were given a training period of 1 month during which they were encouraged to use the GEMs. Training included trailing feed pellets on the ground in front of the GEM and increasing the frequency and amount of dispensed feed-pellets. The measurement period began after the training period and run for 7 weeks from 14 November to 27 December 2013, being the end of spring in this geographic region. The cattle were weighed in the same yards, straight off pasture without a fast, at the start and end of the test period, and were swapped between paddocks mid-way through the test to reduce confounding of results for each sex with paddock and GEM. Cattle management and nutritional characteristics of the pastures over the measurement period are described in Velazco *et al.* 2015.

Figure D5. High and low feed efficiency cattle at Glen Innes being measured for methane production by GreenFeed emission monitoring units in the paddock.

2.3.2 Measurement of over 700 cattle in the Beef Information Nucleus during 70 tests for Residual Feed I (RFI) intake at Tullimba feedlot.

These cattle are commercial cattle provided by cattle breed societies and typically come in groups of 40 head/cohort. They include Angus, Hereford, Wagyu and Charolaise but predominantly Angus. Five batches (total of 548) of Australian Angus and one batch (total 107) of Australian Charolais Beef Information Nucleus herd steers which were undertaking a net feed intake test at the University of New England “Tullimba” research feedlot, near Armidale NSW, were measured for MPR.

Figure D6. GreenFeed emission monitoring units fixed in the feedlot pen where cattle access their main ration form the GrowSafe feed bunks beside; as used of screening industry cattle for feed efficiency and emissions

2.3.3 Measurement of 24 grazing cattle in North Queensland (Charters Towers)

The study site was situated on the “Fletcherview” research station, a 6000ha cattle enterprise supplying commercial cattle for trial purposes located approximately 26km North of Charters Towers in northern Queensland. Two adjoining paddocks 1 and 2 with similar (almost identical) aspect, topography, land type and pasture base were selected and were 29.36ha and 33.67 ha respectively in area with the same estimated stocking rate capacities. Trial paddocks were predominantly black soil with both paddocks containing a basalt ridge and red basalt at the southern end of the paddock. Both paddocks had only one water source/point situated in similar relative positions in each paddock. The paddocks had been grazed prior to the month preceding the trial at which point significant wet season rainfall fell across the entire station including the trial paddocks selected. From this point on and continuing past the trial conclusion, pasture growth was substantial. Much of Queensland including “Fletcherview” in its entirety had been experiencing a severe drought up until this point.

Animals
Subsequent to liveweight assessment 45 red Brahman mixed-sex yearlings were introduced to a paddock containing a single GreenFeed emissions monitoring feeder unit (GEM) 16 days prior to commencement of the trial period. This initial 16 d period was intended to allow acclimation to the GEM feeder and allow selection of animals on the basis of visitation to the GEM unit. Prior to the acclimation period all steers were implanted with a single dose/implant of Compudose® 100 containing 21.1mg oestradiol 17ß per dose via subcutaneous ear implantation. At the conclusion of the acclimation period 24 animals were selected and allocated to treatment by stratified randomization based on initial liveweight and sex with 6 steers and 6 heifers allocated to each of two groups.

Upon selection and allocation to the two groups, GPS tracking collars were deployed on all 24 animals, individual faecal samples collected and animals were returned back to the two selected trial paddocks in their respective groups. Animals remained in their respective groups and allocated paddocks for two consecutive 14 day periods with the exception of a single day separating these two periods when GPS data was downloaded and faecal and blood samples collected. Animals in group 1 were given access to the GEM unit in paddock 1 for the first 14d period and animals in group 2 were given access to the GEM unit in paddock 2 for the second 14d period in a cross-over design experiment so that changes in pasture availability over time would not confound effects of the presence of the GEM unit

GPS tracking collars
GPS tracking collars were assembled based on the methods as described in Allan et al (2013). A commercially available i-gotU GT-600 GPS chipset was used due to its cost, size, ability to power save in a sleep mode, integrated GPS receiver and 64mb flash memory giving it the ability to store some 262,000 positional records. Some alterations to the design described by Allan et al (2013) were made to the GPS collars to suit the purpose of tracking cattle including a more robust IP69 rated polycarbonate enclosure to encase the GPS chip set and battery and a separate counterweight to ensure the GPS receiver in the main enclosure had adequate sky view. The collar material and design also differed to that described by Allan et al (2013) with the addition of a second buckle and use of more robust PVC coated webbing. This allowed the main GPS enclosure to be positioned in the optimal position on the neck of each animal. A 3400mAh rechargeable lithium ion battery was fitted to the chip set to allow for longer duration of operation. The factory push button on/off switch was desoldered off the chipset and replaced with a magnetic reed switch to prevent accidental power cycling. The factory USB plug was also removed and an IP69 rated mini USB panel mount plug was soldered to the chip set and fitted to the polycarbonate enclosure to allow download of data without having to disassemble the GPS chipset and battery enclosure. GPS chipsets were programmed to a duty cycle (wake-sleep-wake) of 300second intervals and if at such a point could not provide a fix would wait until the next scheduled fix for the next attempt.

GPS data analysis
GPS data was initially cleaned and analysed for heat map (point) intensity development and walking distance and speed using the OZ Track web based platform. More in-depth analyses were then performed on the raw GPS data using ArcGIS (ERSI 2006) as described by the methods of Trotter et al (2010).

Greenfeed emissions monitoring
During the initial 16d acclimation period all 45 animals from the original station cohort were given access to the GEM unit to allow acclimatization to its operation. During this period all animals were given access to the GEM unit in the same paddock and the GEM unit was set to allow unlimited food reward delivery upon RFID and proximity detection. Upon commencement of the main trial period (the first 14d period) food rewards were reduced to 3 feeding sessions per day per animal through the day with a maximum of 4 drops of approximately 50g of pellets per feeding session making a total of 600g of pellets per day per animal. Minimum time between feeding sessions was set to 8h, with 4 drops per feeding session and a minimum time between drops within feeding session of 45seconds. A feeding period was defined as the time when the raw data value of the proximity sensor was above 600; this criteria had to be met for 3 or the past 5 seconds before feed was delivered to the animal. The GEM unit was powered on a solar system comprising 2x 250watt solar panels, 2x 225ah deep cycle gel batteries and 1x MPPT solar regulator. As such to reduce power consumption of the GEM unit the exhaust fan was set to automatic and would be turned on when a RFID tag was detected. Once turned on the fan switching off was delayed to 300s post animal leaving the unit. Data was stored on on-board flash storage and was downloaded to the server every hour via nextG/3G telemetry. The GEM unit gas sensors were calibrated prior to the acclimation period and again on day 13, 15 and 28 using pure N_2 as the zero gas and a custom gas mixture comprising 0.1% (1,000ppm) CH_4 and 1% (10,000ppm) CO_2.

Sample collection
At commencement, day 15 and day 29, all cattle were weighed, faecal sampled and blood collected via tail venipuncture. Faecal and blood samples were chilled on ice during collection and immediately frozen down in a -20^{o}C freezer post collection. Blood samples were collected into vacutainers containing lithium heparin.

Sample preparation and analysis
All blood samples were thawed and spun down in a centrifuge at 1800g for 15minutes. Plasma was removed and stored at -20^{o}C prior to analysis. Plasma samples were then thawed and analysed for Calcium and Phosphorus in a Dade behring dimension RXL clinical chemistry system autoanalyser.

Faecal samples will be processed and analysed for alkane and other plant wax profiles as markers to determine and compare nutrient supply using the method as described by Dove and Mayes (2006).

2.3.4 Measurement of 30 cattle during a Grapemarc feeding study (AoTG1-182)

A study was made to determine the effect of different inclusion levels of Grape Marc (GM) on animal performance and daily methane production (DMP; gCH_4/d). A 3x3 Latin Square experiment was conducted with approval from the Animal Ethics Committee (AEC) of the University of New England-Australia (AEC 13/147)

Location
The experiment was conducted at the University of New England's Tullimba beef cattle feedlot farm situated North of New South Wales, Australia (30^{o}20'S; 151^{o}12'E). The average annual rainfall in the region is 805mm during summer that runs from October to March. This experiment was carried from April to August 2014 and therefore during winter when humidity was high (80-85%) and temperatures very low (Kaara, Riaz, & Graeme, 2010).

Figure D7. Purebred Brahman cattle in study of methane mitigation impacts of processed grapemarc.

Cattle and diet
Thirty steers and Heifers (Angus and Brahman) of different live weights (LW) ranging between 332 to 412kg (365±23kg) and 316 to 416kg (373±32kg) respectively were allocated to 3 groups. Each group was composed of 5 Angus steers and 5 Brahman heifers aged 22 -26 months, with each group housed in a separate open 15m x 10 feedlot pen. Only 10 animals per pen were used to avoid competition within the pen for access to the feed bin and minimize risk of animals reducing feed intake. The choice of different breeds in this experiment was because of the interest to capture any differential response in methane emission and performance response to GM for cattle of different growth pattern. In this study breed and sex were confounded but there is no record of sex affecting DMP or methane yield, so any differences between Brahman and Angus can be considered breed effects.

Prior to providing the treatment, the diet of the cattle used in the experiment was adjusted to basic feedlot finisher ration, based on barley and maize silage, over a period of one month. One Brahman was removed due to potential laminitis during adaption to diet The Dry Matter (DM) contents (in g/100g equivalent) of the finisher ration were analyzed in the Wagga Wagga Chemistry Services Laboratory (Australia) and results include dry matter (90.2), neutral detergent fibre (18), acid detergent fibre (8), crude protein (12.3), dry matter digestibility (86), digestible organic matter in the dry matter (84), inorganic ash (5), organic matter (95), metabolisable energy (13.5) and crude fat (3.8).

The diet of each group of animals was changed to isoenergetic experimental diets (treatment) containing 0%, 10% and 20% Grape Marc feed.

2.3.5 Application with finishing cattle with or without nitrate blocks supplied (100 hd/group; AoTG1-182)

In association with the Australian Country Choice organisation, a trial was established at ‘Dungowan’ Augathella in Queensland’s central west. Cattle were offered the same treatments as are offered at the Burleigh (Richmond Qld) site for this project. These are a urea multi-nutrient block, a nitrate based multi-nutrient block (30% Bolifor) and either free choice of urea, S, P single–nutrient blocks, or the same N,SP blocks that all also contained 30% Bolifor.

An automatic 4 way drafter mounted on a walk over weigh unit directs cattle to the correct supplement based on their eartag.

2.3.6 Demonstration of sheep GreenFeed units in grazing environment

This section is confidential

2.4 Developing validation procedure for GEMs and optimised use protocols

The required outcome here was that a standard international protocol drafted for use of GEM units to verify emissions mitigation in sheep and cattle. Meaning a test protocol for comparing GEM and RC units be devised through international collaboration. This was done by email discussion between UNE, the US manufacturer and scientists using GEM units in the UK and New Zealand.

To more effectively design experiments in which GEM units were to be used for mitigation, we also addressed key aspects of experimental design around how many samples are required to conduct comparisons of specific power. These included:

- How many measures are required to quantify a treatment mean with specified confidence,
- How many measures are required to quantify an animal's methane phenotype with confidence,
- How accurate are EBVs for methane traits based on GEM measures

The detailed methods (largely statistical) by which these were achieved are described below.

2.4.1 How many measures are required to quantify a treatment mean with specified confidence,

The precision of estimated DMP or MY values, when feed intake data is not available, depends on the inter-play between the different sources of variation and the numbers of replicates within each component. Taking our DMP estimate based on spot emission data as an example, we first fitted a 'base' mixed model with no fixed effects and 'animals' and 'days' as the random effects (to estimate their variance components). As listed in Table D1, the residual variances were large (vs. their respective standard errors), however, 'days' do not appear to be a simple random effect, as there is an increasing trend in DMP over time. Table D1 also shows the variances for a second mixed model where the observed trend is accounted for by including a linear term as the fixed effect.

Table D1. Variance components (s.e.) from the general linear mixed models of MY and MP

	DMP		MY	
Fixed effect	Nil	Days (linear)	Nil	Days (linear)
Residual (between-measures; within-animals and within-days)	1689 (45)	1687 (45)	14.84 (0.40)	14.84 (0.40)
Animal	160 (52)	164 (53)	2.69 (0.83)	2.67 (0.83)
Day	935 (176)	349 (71)	6.51 (1.23)	5.48 (1.05)

Removing the trend in days reduced the estimated variance components for days from 935 to 349 for DMP and 6.5 to 5.5 for MY. Even when de-trended, the variance components for days are notably larger than those for animal. Other cattle methane studies (e.g. Blaxter and Clapperton, 1965; Boadi *et al.*, 2002; Harper *et al.,* 1999; Pinares-Patino *et al.*, 2003; Vlaming *et al.*, 2008) indicate that between day variance in DMP is more likely to be closer to our lower value (i.e. when effect of our linear trend is removed), so we used the between day variance value of 349, rounded to 350, in a power analysis of experimental designs for DMP estimated from spot measurements.

For the power analyses of DMP, we rounded the linear trend values to 1700 for the residual variation and 160 for the variance components for animals. The power analysis investigated the precision of the estimated mean using the 95% two-tailed confidence interval, taking the variance formulae in Cox and Solomon (2003) as shown below.

$$\text{s.e. (mean)} = \text{sqrt}\left[\sigma^2 / (n_a.n_d.n_r) + \tau_a / n_a + \tau_d / n_d \right],$$

where

σ^2 is the residual variance,

n_a, n_d and n_r are respectively the numbers of animals, days and replicates, and

τ_a and τ_d are the variance components for animals and days respectively.

The targeted precision was 5% of the estimated 64d mean DMP or MY. Hence we expect 95% power for any future experiments with combinations of numbers of animals, days and replicates where this target is met. Figures 6-7 and 6-8 show these patterns for DMP and MY respectively, taking our observed number (2) of replicate spot measures per animal per day.

2.4.2 How many measures are required to quantify an animal's methane phenotype with confidence,

Two data sets (grazing and feedlot) were used to calculate the minimum number of spot flux measures needed to phenotype the true average CH_4 emissions of an animal as required to develop DMP estimated breeding values (EBVs). DMP was estimated from multiple 3-5 min spot measures of methane flux made by the GEM system using 24 cattle. The analysis was based on an acceptable margin of error (MoE) for sampling, a level of confidence to be associated with the final estimates, and an estimated coefficient of variation for each particular sample. MoE is the maximum permitted deviation of the estimate from the true mean. These calculations assume the confidence level for sampling would be 90% (i.e. the measured value of DMP should be within 10% of the true value). DMP estimates from cattle were estimated by GEM while cattle grazed pastures (173 gCH_4/d) then again when they were feedlot finished (DMP = 142 gCH_4/d). The MoE for each individual methane measurement was chosen as ± 5-10 gCH_4/d. Measurement errors expressed as a percentage of the means, when MoE = 10 gCH_4/d were therefore, 100*(10/142) = 7%, and 100*(10/173) = 6% for feedlot and pasture respectively. Sample sizes, calculated as follows, required assuming a margin of error and a desired level of confidence:

$$\text{Sample size (N)} = (z^2 * CV^2)/(MoE/\square)^2$$

where:

z is the value associated with the chosen confidence interval,
CV was 40% (feedlot) or 30% (pasture), and
MoE/μ is the ratio between the margin of error and the mean.

2.4.3 How accurate are EBVs for methane traits based on GEM measures

To study the effect of number of days and measures per day on the precision of phenotypes estimates and EBVs for DMP and MY, the 95% two-tailed confidence interval was estimated from the variance estimates reported by Cottle et al. (2015), using the variance formulae in Cox and Solomon (2003) as shown below:

Standard error (mean) = sqrt [σ^2 / (n_a.n_d.n_r) + τ_a / n_a + τ_d / n_d],
where:

σ^2 is the residual variance,
n_a, n_d and n_r are respectively the numbers of animals (1), days and replicates, and
τ_a and τ_d are the variance components for animals and days respectively.

The confidence intervals for EBVs were estimated as ±1.96 * $\sqrt{((\sqrt{(1-h^2)}*\sqrt{V_A}) + V_E)}$,
where:

h^2 is heritability,
V_A is additive genetic variance, and
V_E = environmental variance ($(1-h^2)*V_P$).

3. Results and discussion

3.1 Validating GEM estimates of Daily Methane Production against respiration chambers

The fundamental query is whether collection of multiple short-term (3-6min) measures of emission can accurately quantify the daily methane production of an individual or group of individuals. If not hen the GEM is unlikely to have a future in emission research; if so, then there will be a requirement to work out how to appropriately use the GEM system to obtain accurate estimates with sufficient precision to be useful in accurately describing the average DMP of a group, or the DMP of an individual.

The results for the 2 multi-period studies used in this assessment are shown below (Table D2).

Table D2. Dry Matter Intake (DMI), daily methane production (DMP) and methane yield (MY) measured by open circuit respiration chambers (RC), by GreenFeed Emission Monitoring units delivering supplement (GEM-s) or delivering water (GEM-w) as attractants.

Experiment	Technique	DMI (kg/d)	s.e.m.	DMP (gCH_4/d)	s.e.m.	MY (gCH_4/kgDMI)	s.e.m.
1	RC	9.30_a	0.92	215.8_a	9.2	23.71_a	1.01
	GEM-s	9.27_a		208.6_a		22.71_a	
	GEM-w	9.27_a		105.7_b		11.40_b	
2	RC	8.98	0.25	198.3	3	22.14	0.42
	GEM-s	9.3		214.6		23.83	
Combined analysis	RC	9.02		206.7	3.5	22.9	0.45
	GEM-s	9.13		212.1		23.24	

[a,b] Values within a column with different superscripts differ significantly

In summary no significant difference was found between GEM estimates and RC measures of DMP in either experiment (Table D2). These calculations used a value for DMI that was the average of the day of measurement and the day before, in recognition that DMI up to 2 days before has been shown to affect DMP in sheep. These results gave confidence that GEM units could be used to estimate DMP accurately and would have application in the field.

A further comparison (conducted under a CSIRO CLUSTER project) also confirmed the alignment between GEM DMP estimates and both the IPCC predicted DMP (6.5% GE intake) and that determined in RCs (for the same cattle consuming paired intakes of the same feed).

During NLMP it has become apparent that while RC offer a precise and generally accurate measure of DMP, even a 'perfect' measure of emission over 24h does not completely describe the daily emission from an animal, as the repeatability of sheep RC data shows a high repeatability across consecutive days but not across measurement made 14d or longer apart. This has created a strong interest in how many measures and how spread out must they be to accurately describe the methane phenotype (DMP or methane/kg DMI) of an animal.

An important observation was made about the between-day variance in GEM estimated DMP and MY, versus the between animal variance (Table D2). This study (based on approximately 60 cattle measured over approximately 70d) shows that between day variance (even with linear adjustment) exceeds between animal variance. As a consequence, the requirement for sampling across the diurnal variation in emission rate (example in Figure D9) is not necessary. Because variation in emission day-to-day is so high, it is as valid to collect across more days as across the diurnal variation within a day.

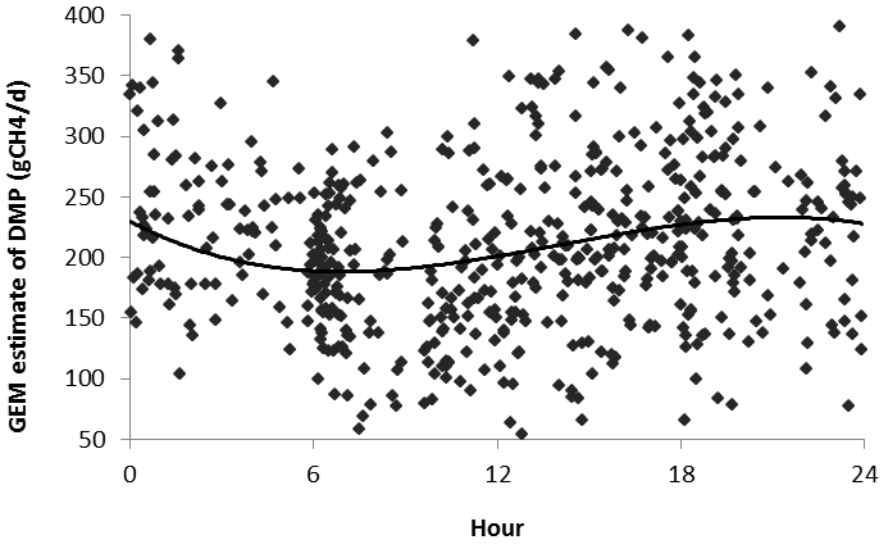

Figure D9. Distribution of daily methane production estimates (DMP) and visitation pattern (points) and cyclic diurnal pattern for DMP (line) for all cattle at the GEM-supplement during periods 1 and 2. Each dot corresponds to an individual visit, and daily CH_4 production is expressed in gCH_4/head/d at the time of the visit.

3.2 Development and validation of the sheep GEM units

This section is confidential

3.3 Application of GEM units in on-farm demonstration and measurement

As previously described, the GEM units have been applied within the following measurement and demonstration environments:

3.3.1 Measurement of 60 cattle of divergent generic merit for RFI while grazing (at Glen Innes NSW) and while finishing in a feedlot (Tullimba NSW)

Emissions of GHG gases (CH_4 and CO_2) by individual cattle in the pasture and feedlot test phases were determined from multiple short-term breath measurements using US-manufactured Greenfeed emission monitors (GEMs; C-Lock Inc., Rapid City, South Dakota). Only 34 head had sufficient valid records for GHG emissions for all test phases and only results for these cattle are presented. The cattle were older and heavier across the phases of the experiment, but their feed-intakes and CH4 production rate (MPR) did not increase proportionally due to differences in how the cattle were fed across the phases (Table D4). Methane emissions (MPR and methane intensity, MI: MPR/ADG) were highest when the cattle were grazing on medium-digestibility pasture. In the feedlot, on ad-libitum access to a higher-digestibility ration, MI was halved compared to that at pasture and MPR was not increased. Methane yield (MY; MPR/DMI) was lowest in the feedlot phase, and increased as feed offered was restricted to 70% of that consumed individually over last 2 weeks in feedlot for the chamber grain test, and was higher again on the restricted (to just above expected maintenance) chamber roughage test. Repeatabilities of methane emissions by individual animals relative to their DMI calculated as MY and two measures of residual methane production (RMPJ and RMPR) between the feedlot test and the restricted grain test, and between the restricted grain test and the restricted roughage test were moderate (*r*-values ca. 0.4), and stronger between the feedlot test and the restricted roughage test (*r*-values ca. 0.6), and provided evidence that methane emissions relative to feed consumed are at least moderately repeatable over the testing regimes of the experiment. Carbon-dioxide production rate (CPR) was positively correlated with DMI in the 3 test phases where DMI was measured. This offers the possibility that CPR could be used as a proxy for feed-intake to adjust MPR for variation in DMI when DMI cannot be measured. Higher Midp-EBV-400dWT was associated with heaver WT of animals across the 4 phases of the experiment and with higher GHG emissions in the feedlot: higher MPR, CPR, MI, RMPW and RMPR. Lower Midp-EBV-RFI (genetically higher feed efficiency) was associated with heavier WT at pasture, and faster ADG, lower DMI, lower RFI and lower FCR, but no change in MPR in the feedlot.

Table D4. Means for feed metabolizable energy content, mid-test weight, dry-matter intake and selected methane emission traits across the four test phases of the experiment for the 34 Angus cattle with data analysed.

	Pasture (Greenfeed)	Feedlot (Greenfeed)	Chamber grain	Chamber roughage
ME (MJ/kg DM)	9.2	13.5	13.5	9.0
Feed offered	grazing	ad libitum	restricted (70%)	restricted (1.2xMtn.)
MidWT (kg)	337	464	524	556
DMI (kg/d)	-	12.5	7.2	7.7
MPR (g/d)	175	142	109	147
MI (g/kg ADG)	216	103	-	-
MY (g/kg DMI)	-	11	15	19

3.3.2 Measurement of over 700 cattle in the Beef Information Nucleus during 70 tests for Residual Feed I (RFI) intake at Tullimba feedlot

These studies were conducted in association with BCCH 6310 (Genetic technologies to reduce methane emissions from Australian beef cattle.) and the data from these measurements is reported in that report. For the purpose of this report however, these studies provide an opportunity to properly cost the annual operation of the GEM units and this is provided below.

Budget to maintain 6 GEM units @ Tullimba for a period of 12 months		
Item Cost		
Items	**Unit**	**Unit Cost**
Internet Cost	GEM Month	$ 35.00
GF Pellets	Kg	$ 0.55
Travel	Km	$ 0.81
Labour	Hour	$ 38.00
Calibration Gas Replacement -Nitrogen	D-Cylinder	$ 20.00
Calibration Gas Replacement -Methane M	E-Cylinder	$ 612.00
Propane-		$ 12.00

Variables	
Travel to site (=km return)	120
Number of GEM Units	6
Pellet Consumption head/day (kg)	0.6
Number of cattle/pen	70
Duration (days)	365
Number of Pens	3
GEM Units/Pen	2

Budget to maintain 6 GEM units for 1 year at Tullimba			
1	Feed Pellets for GEMS	$ 25,294.50	210 cattle eating 600g/h/day @$0.55/kg
2	Internet Cost	$ 1,277.50	At Cost
3	Travel	$ 5,068.29	1 trip/week @ 120km
4	Labour	$ 7,925.71	1 X 4hours/week @ $38/hour
5	Calibration Gases	$ 1,896.00	1 cylinder of each cal. gas/2 GEM units/year
6	Miscellaneous - Filters Repairs etc.	$ 2,000.00	
	Total	**$ 43,462.00**	

3.3.3 Measurement of 24 Brahman/Senepol/Charolais X steers during a trial to test feed-use efficiency of feedlot cattle by replacing Urea with Nitrate.

GPS tracking collar data collected longitude and latitude referenced data points time and date stamped which allowed identification of spatial location with temporal references. In excess of 150,000 referenced detections were logged via the 300 second logging interval for the 24 collars deployed. Of these detections 134,722 were filtered into the final data set for deeper analysis. Initially this data was analysed in Oz Track platform to calculate mean daily step length, mean daily step speed and distance travelled. Data was analysed in 48hr days paired with each day overlapping the next. GPS trajectory intensity maps were created using this data and group by period images of these maps are presented in figures D10-12.

Trajectory intensity maps for both groups visually indicate a slight modification in grazing location, trajectory and intensity (time spent in a specified area) and paddock use when the GEM unit was present compared to when it was not. Figure D10 represents the trajectory intensity derived from the GPS data for group 1 when the GEM unit was present in the paddock. Less high intensity patterns were observed on the map in figure 10 when the GEM was present, in contrast to figure D11 which shows comparatively a greater number of more intense spatial presences in areas of the paddock when the GEM unit was not present. That is, there was no indication the GEM caused animals to congregate, but rather the opposite appears to have occurred with the animals remaining more spread out over the paddock area. Similarly figure 5 represents group 2 in the second period when the GEM unit was present in paddock 2 and again indicates similar, less intense use of less areas of the paddock (ie animals more spread out), similar to that observed in figure D10. Again figure 12 shows similar grazing pattern to that represented in figure 13.

Figures D10 and D13 also indicate that when the GEM unit was present animals showed less grazing preference and possibly a more even use of the paddock as indicated by a more even spread of trajectory intensity.

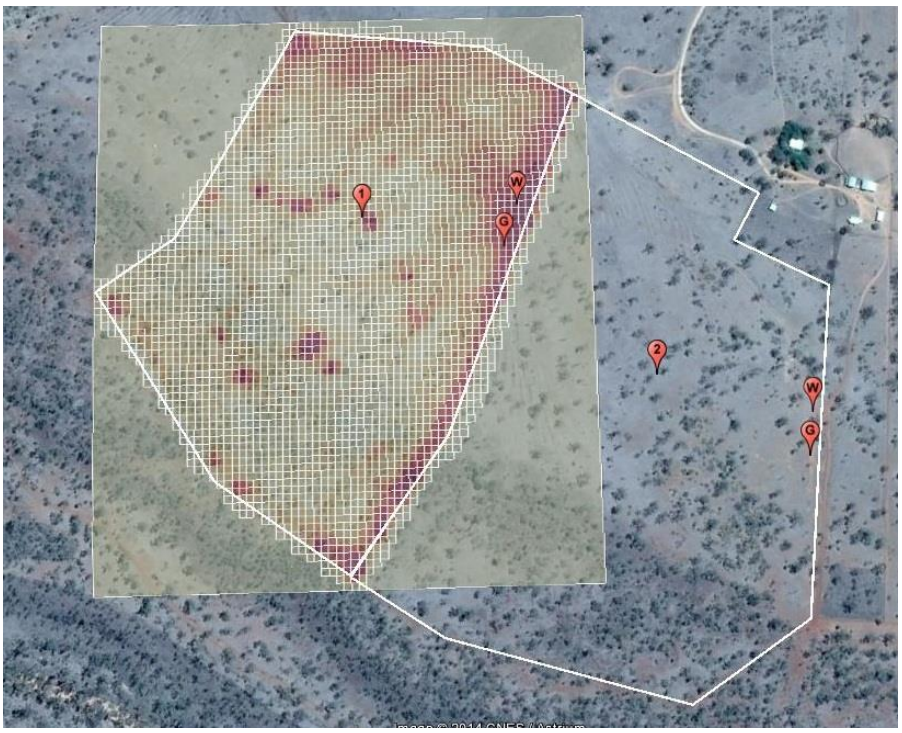

Figure D10. Group 1, period 1, paddock 1 GPS trajectory intensity map – GEM unit present at (G), Water at (W). Yellow to red shading indicates increased intensity of presence in that area. Grid size is 10m.

Figure D11. Group 1, period 2, paddock 1 GPS trajectory intensity map– No GEM unit present at (G), Water at (W). Yellow to red shading indicates increased intensity of presence in that area. Grid size is 10m.

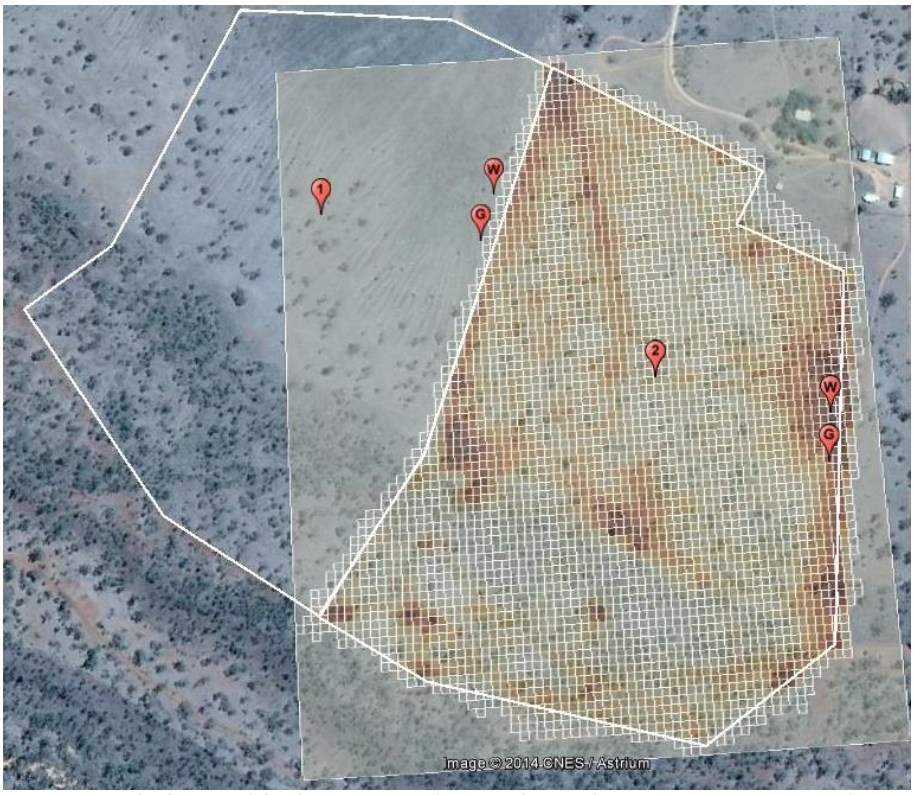

Figure D12. Group 2, period 1, paddock 2 GPS trajectory intensity map – No GEM unit present at (G) in paddock (2), Water at (W). Yellow to red shading indicates increased intensity of presence in that area. Grid size is 10m.

Figure D13. Group 2, period 2, paddock 2 GPS trajectory intensity map – GEM unit present at (G) in paddock (2), Water at (W). Yellow to red shading indicates increased intensity of presence in that area. Grid size is 10m**.**

Statistical analysis of animal location is still required as is analysis of attributes of animal movement. Equivalence testing has begun on the animal movement data but is incomplete. Crude analysis of variance shows no effect of the presence of the GreenFeed (GFeed) on the distance moved bay cattle between positioning (typically 5 minutes) speed of movement of cattle (0.0273 km versus 0.0273 km for no GEM: P>0.05). Nor did ANOVA reveal any effects of GEM unit on the average travel speed of cattle (0.235km/h v 0.253km/h), however equivalence testing indicates to the contrary. What is certain is that there was significant effect of period on total distance walked by cattle, being significantly higher in period 1 (83.4 km/14d v 80.6 km/14d in period 2; Table D5).

Table D5. Fitted means for rate of movement (typically km between location times; SL), average speed (km/h), and total distance walked by cattle over 14d when no GreenFeed unit was present (GFeed =1) in the paddock or not (GFeed = 0). Bold figures indicate significant effect (P<0.05)

	--SL(km/5min)--		------km/h------		------km/14d-----	
GFeed	Mean	SE Mean	Mean	SE Mean	Mean	SE Mean
0	0.0282	0.000380	0.2527	0.007488	82.7815	0.806651
1	0.0273	0.000418	0.2348	0.008244	81.1760	0.888093
Herd						
1	0.0270	0.000388	**0.2365**	0.007656	**77.3340**	0.824780
2	0.0285	0.000410	**0.2510**	0.008088	**86.6235**	0.871283
Period						
1	0.0277	0.000388	0.2483	0.007656	**83.3747**	0.824780
2	0.0278	0.000410	0.2392	0.008088	**80.5828**	0.871283

3.3.4 Measurement of 30 cattle during a Grapemarc feeding study (AoTG)

This study generated liveweight and feed intake data as well but for this purpose only methane data is shown (Figure D14). Inclusion of grapemarc significantly reduced methane emission but only when included as 20% of the ration, not 10% inclusion.

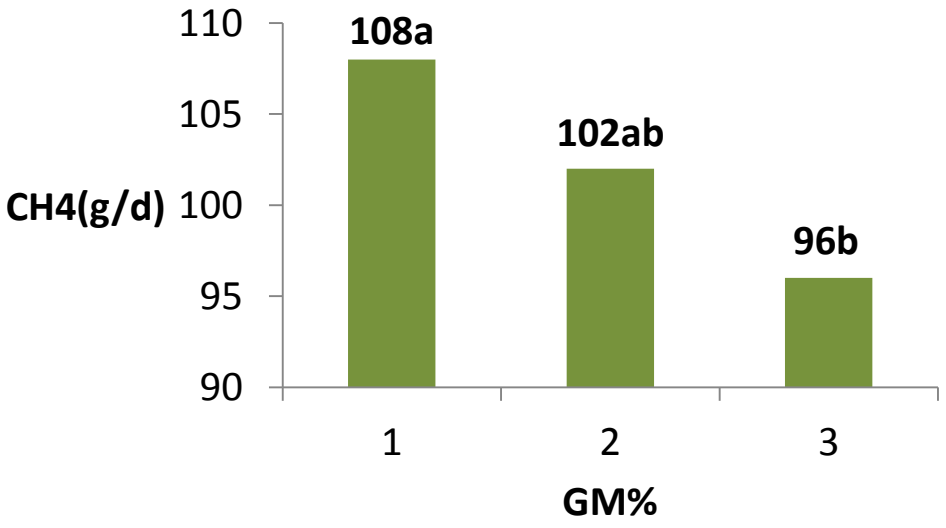

Figure D14. Daily methane production of Angus and Brahman heifers (combined) offered feedlot finisher rations containgin10% or 20% ensiled grapemarc.

3.3.5 Application with finishing cattle with or without nitrate blocks supplied (100 hd/group; AoTG1-182)

This site at Augathella Qld is being run in association with Country Choice Australia, with cattle only accessing the GEM throughout May 2015 and no data is yet available. (Figure D15).

Figure D15. Greenfeed unit operation (solar powered) at Dungowan, Augathella May 2015.

3.3.6 Demonstration of sheep GreenFeed units in grazing environment

This section is confidential

3.4 Developing validation procedure for GEMs and optimised use protocols

3.4.1 How many measures are required to quantify a treatment mean with specified confidence,

Assessment were made of how many short term (GEM) emission measures must be taken to (a) detect a mitigation effect of a specified size between groups of cattle and (b) how many samples/animal are required to accurately define the methane phenotype (DMP or MY) of that individual beast. Findings of these assessments are described below.

We wished to know how many 'spot' (short-term 3-6 min) emission measures were required to define a treatment mean with a specific level of confidence. Data for 70d of emission measures from 60 cattle in the feedlot were analysed for variance structure. The true mean DMP of an animal in the group was approximately 140g CH_4/d (being the average over 70d of measurement) and we wished to know how many days of measurement would be required to provide an estimate of DMP that we were 95% confident was within 10% of the true (70d mean) or within 5% of this true mean. It was assumed cattle accessed the GEM unit twice daily in this modelling. As shown in Figure D17, an estimate of DMP that was within 10% (14g/d – the horizontal grey line) of the true mean required measurement of only 10 animals for 20d, or 20 animals for 10d. Estimating DMP with 95% confidence that the estimate was within 5% of the true mean however could not be achieved with only 10 animals and required 20 animals be fed for 100d.

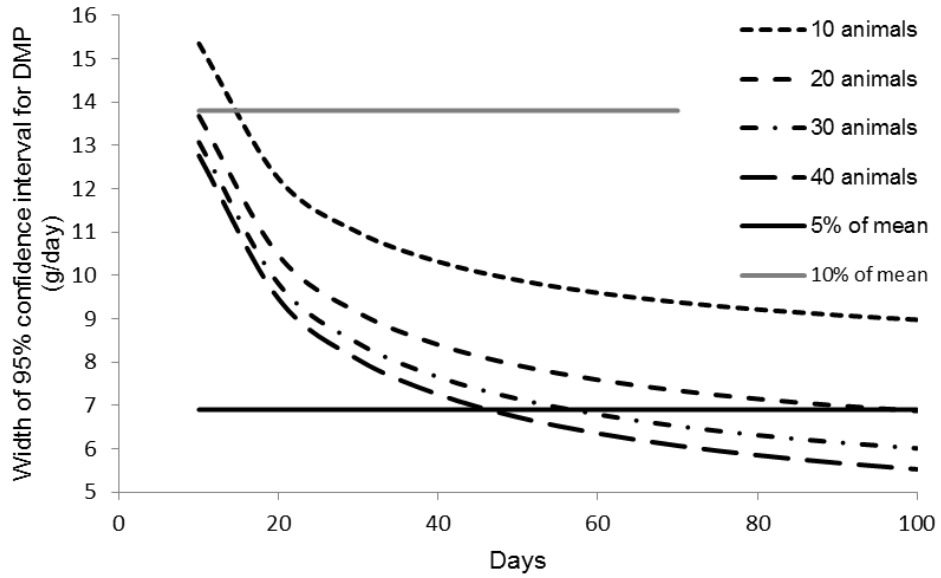

Figure D17. Estimated width of 95% confidence intervals (either side of mean) for daily methane production vs. numbers of days, for different numbers of animals (dashed lines) and the targeted 5% of the observed mean (solid black line) or 10% (solid grey line). Two spot measures of methane production rate per day are assumed

A similar approach was used to estimate the number of animals and days of measurement required to quantify the methane yield (g CH4/kg DMI; Figure D18). These figures suggest that in studies of genetic merit (where 20 progeny/sire are often used), it will be possible to get an accurate sire phenotype based on normal progeny groups.

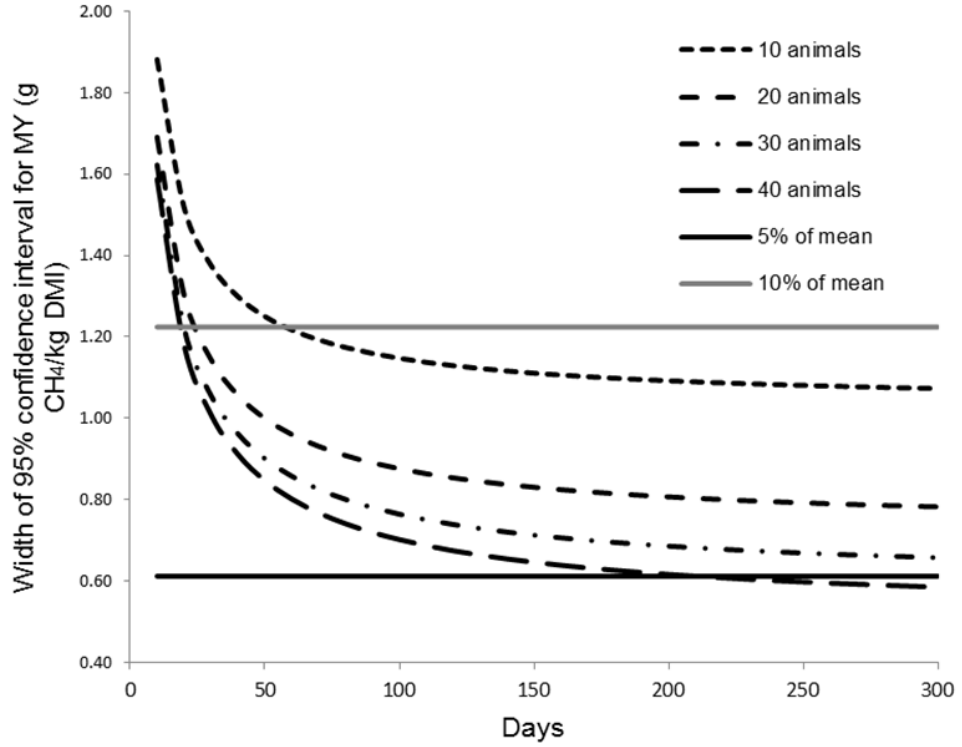

Figure D18. Estimated width of 95% confidence intervals (either side of mean) for daily methane yield vs. numbers of days, for different numbers of animals (dashed lines) and the targeted 5% of the observed mean (solid black line) or 10% (solid grey line). Two spot measures of methane production rate per day are assumed.

3.4.2 How many measures are required to quantify an animal's methane phenotype with confidence,

An alternative approach was then taken to assess the sampling protocol to quantify an animal's phenotype. The analysis based on an acceptable margin of error (MoE; Table D7) shows that (for example) to be 95% confident that the average DMP estimate is within 10g of the animal's 70d mean DMP, requires 54 emission measures be collected from that animal.

Table D7. Number of short-term GEM measures required to estimate the DMP phenotype of an individual animal (g CH_4/day) with a specified margin of error and with a defined confidence using feedlot and grazing data sets

	Confidence interval (%)			
MoE (gCH_4/d)	70	80	90	95
Feedlot data set				
5	61	93	153	217
7.5	27	41	68	97
10	15	23	38	54
Grazing data set				
5	54	81	134	190
7.5	24	36	60	85
10	13	20	34	48

3.4.3 How accurate are EBVs for methane traits based on GEM measures

In seeking to determine the accuracy of Estimated Breeding Values (EBV) that may flow from such measures further analysis was undertaken and the number of days of measure (at 2 measures/d) determined for 95% confidence in the EBV (Figure D18). It is apparent that the 95% confidence interval is large (~27g/d and 3.5 g CH4/kgDMI) for the EBVs of DMP and MY.

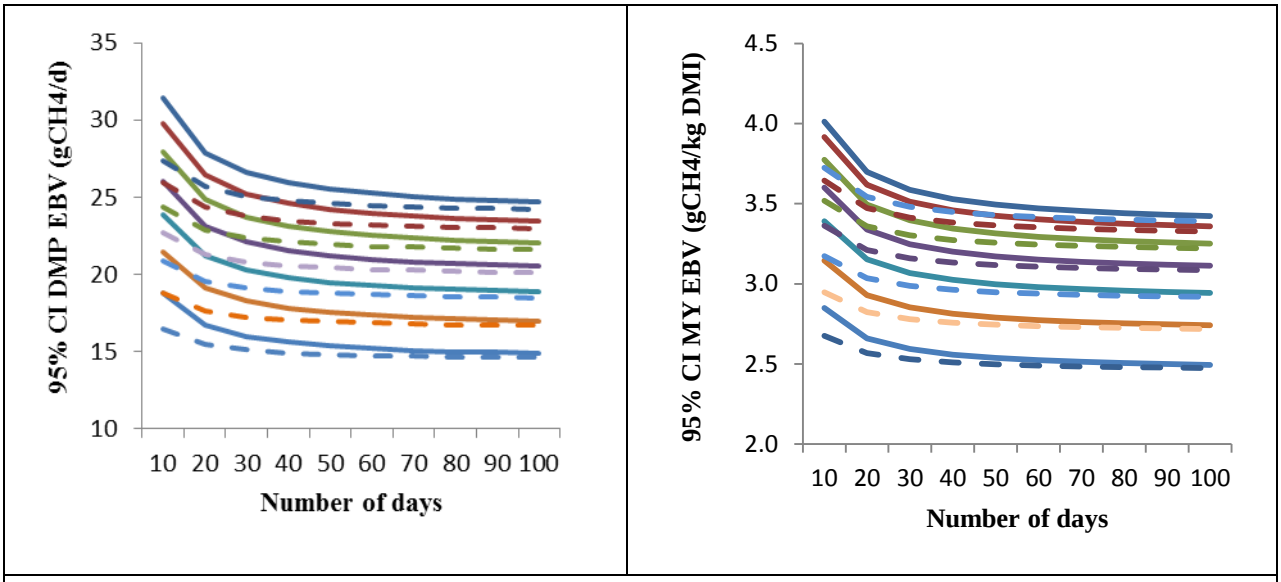

Figure D18. Estimated width of 95% confidence intervals of a) DMP EBVs and b) MY EBVs (either side of mean) vs. numbers of days with 2 measurements / animal / day (solid line) or 10 measurements / animal / day (dashed line). Heritability from top to bottom: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7. (Cottle et al., 2015 submitted)

The 95% confidence interval for DMP EBV estimates was ±25 gCH_4/day and for MY EBV estimates was ±3.5 gCH_4/kg DMI, assuming a heritability of 0.26 for DMP and 0.23 for MY (Herd *et al.* 2014b). Increasing the number of measurements / animal / day or number of days of measurement, (i.e. total number of measurements), had little impact on the precision of EBVs. The confidence intervals are about 20% of the mean values for DMP (~150g CH_4/day) and MY (~13g CH_4/kg DMI) each side of the mean, which should be of adequate precision for use in industry via breeding schemes such as BreedPlan.

4. General discussion

Overview of key findings:

Regarding accuracy, we have found no difference in estimates of daily methane production (DMP) by cattle GEM units relative to RCs. This is supportive of the use of cattle GEMs for quantifying emissions and verifying the level of mitigation that may be achieved. We were able to demonstrate that between day variation in DMP (estimated by GEM) was larger than between-animal variation which had 3 implications for measurement. Firstly the fear of biasing the DMP estimate by cattle accessing the GEM either before or after feeding is ill founded. More measures/d do not improve the variance of the measure, indicating that the focus should be on more days of measure, not more measures/d. Secondly, this is advantageous as it means the cattle do not have to repeatedly access the unit every day which could lead to supplement becoming a big part of their diet distorting their natural emission level. Additionally, few visits/d will further reduce the risk that accessing GEM will modify the grazing behaviour and intake of cattle (tested and found non-significant tin this project). The number of days and visits required is consistent with the approved feeding program for cattle being assessed for Residual Feed Intake (RFI) determination (70d test) meaning DMP, MY and RFI can all be measured concurrently.

Regarding practicality, we summarise our knowledge on use of the GEM as follows:

- Accessing the GEM units and having emissions measured is a voluntary activity for cattle and sheep. We cannot (or at least have not), tried to force animals in or hold them at the GEM to ensure measurements are made.

- Approximately half of the animals will choose to enter the GEM to receive pellets; this proportion is not consistently higher in either feedlot or paddock.

- Inclusion of an attractant (aniseed) appears to help recruitment to the units.

- The proportion of animals accessing the GEM increases with prolonged exposure

- The key to high recruitment is to provide liberal access to the unit and to supplement when animals are first exposed, and have all distractions (eg. Lights, bells and fan) turned off.

- Being able to trigger pellet release remotely is critical for recruitment in remote locations.

- The cameras mounted on GEM units allows the operator to monitor GEM access problems (eg more than one animal at a time in the measurement area) and act accordingly.

- The streaming, via the C-Lock server, of the real-time readings for a variety of parameters including airflow, head proximity, battery voltage, gas concentrations, wind direction and wind speed for individual GEM units, enables the operator to make fast, accurate decisions on maintenance issues (filter changes, calibrations, battery maintenance etc)

- Having an experienced GEM operator on-site during the early days of access to the unit makes a large difference to success of the operation.

- Perhaps reflecting temperament, our assessment is that recruitment is highest for docile dairy cows (NZ collaborator experience), intermediate for Australian ‘southern beef’ where cattle have some regular handling and least in remote locations where cattle are not routinely handled. Cattle

in remote locations live in quiet 'un-mechanised' environments so the noise of the fan and of the motor driven supplement dispenser can frighten cattle (but ultimately become an attractant).

- The units and supporting solar panels and cattle panels are heavy and ideally a pair of units and associated materials would be moved on a light (4t) truck rather than a utility and trailer.
- We have developed capacity to make the units run on satellite (rather than 3G network) but have not had cause to implement this yet.
- The manufactures have implemented our suggestion to incorporate a gas auto-calibration system, reducing the manual maintenance requirements especially in remote locations.
- The manufacturers would always encourage great care in moving the units and damage due to transport is unlikely to get much sympathy (or replacement parts). Moving units can work connections loose as has been our experience.

Regarding the sheep GEM; the construction and hardware operation of the sheep GEM units is far superior to the original cattle GEM units. This reflects a better design, with all sensors and electronics being located through an easy to access service panel, rather than being buried beneath the feed tray as in cattle units. Also the quality of the electronics appears higher, with no problems in motherboards, RFID detection, wireless connections or pellet delivery mechanisms. Certainly part of the easier run with the sheep GEMs has come from technical staff having a very high level of familiarity with all components and procedures after 3 years work on cattle units, but the units themselves are much improved.

The 2 studies comparing RC and sheep GEM estimates of DMP gave differing results, with Experiment 1 showing the GEM estimate of DMP was significantly higher than that determined in chambers subsequently. This was associated with a higher methane yield as estimated by the GEM. The experiment was however repeated because of hot conditions during experiment 1. DMI used in estimating MY (an average of the DMI on the day of measurement and the day prior) did not differ between GEM and RC, nor did the intake on the day of RC and the ad-libitum intake over the preceding day. The chambers were very hot however due to an unseasonal period of hot weather (local weather 26.1 +/- 4.2^{o}C max over experiment 1). The experiment was therefore repeated and no effect of measurement method was found on DMI, DMP or MY. When data from the 2 studies were pooled and a combined analysis run, sheep had a higher DMP as determined by GEM than RC. While this was in part because of difference in intake (1.64 v 1.55 kg/d); there was also a difference in MY between methods. While it is inappropriate to draw conclusions when 2 studies give differing findings, it is apparent that both methods identify changes in emissions across periods but that more comparisons are needed to provide confidence in the accuracy of DMP derived from the sheep GEM.

There are also some critical evaluation questions relating to the sheep GEM discussed below and presented here as part of the final milestone for the project:

What are the key implications of the results (Sheep GEM) obtained?
The estimate of DMP provided by the sheep GEM unit differed from that measured by RC on one occasion but not on a second assessment; however the pooled data indicates significant difference between methods. Since the previous report (when only 1 test has been conducted), an error in the post-collection data processor was found and this process has been rigorously interrogated. The fact that CO2 recovery is near 100% suggests that if errors in DMP are being made, they are likely to be errors in the processing stage, not the data collection stage.

The consequence is that we cannot yet be confident that sheep GEM units rightly quantify DMP of sheep but we are confident that sheep will access the GEM in a paddock environment and that the equipment itself is robust and functional for prolonged use (at least 1 month continuous running under solar power).

What are limitations?
The principal limitation is lack of confidence in the accuracy of the DMP estimates generated by the sheep GEM, with our 2 stue4is the only 2 conducted in the world.

What are the main outcomes?
The main outcome is we have shown the Sheep GEM to be practical and functional in terms of its hardware design and data collection protocols. We have also shown that the Monensin does not reduce daily methane emission from sheep in keeping with what has been found for dairy cows. It is also apparent that HQ156 does not eliminate protozoa from grazing sheep so is not a potential defaunation tool when delivered by this means (low regular intake via supplement).

What questions remain either unanswered or partly answered?

- With one study showing GEM overestimated DMP relative to chambers, and another finding no difference, it is important to ascertain the scale, frequency and reason for the difference in DMP estimates. We have comprehensively assessed all data calculations for both studies reported here and further studies are likely to be required.

- Even though accuracy of DMP estimates remain in question from sheep GEMs, the tool can still be sued to detect relative differences in DMP between treatment groups and this be applied in such tests as methane production by sheep grazing nitrate-blocks that has previously only been quantified in respiration chambers.

Significance of Overall GEM findings

This project was principally developing, validating and demonstrating the GreenFeed Emission Monitoring System as a tool for mitigation research, perhaps extending as far as demonstrating the process of its use in collecting field data for genetic and nutritional mitigation in the field. It was not specifically about developing a mitigation strategy as many projects were, but its impact, relevance and implications are explained below.

The start of NLMP was the start of the livestock industries having an interest and possible need for on-farm mitigation. As part of this process we have seen novel approaches to emissions measurement tried in search of rapid, simple and cheap means of quantifying enteric methane production. These include:

- Measuring total emission over a 30min – 8h period by confining animals in a restricted area (Charmley 2015 unpub) or in a sealed box (Goopy 2013)

- Measuring breath emissions over multiple 3-6 min periods by GreenFeed (2015)

- Measuring breath concentration during milking (and imputing flux rate; Garnsworthy et al. 2012)

- Measuring breath plume methane concentration by laser and imputing flux rate.(Chagundra et al., 2010)

The attraction of the GEM approach is that it does accurately measure emission rate (albeit over a short time) and a long term emission rate can reasonably be expected to be derived from repeated measures spread over a longer timeframe. In comparison, measurement of animal in confinement (be it around a paddock water point or sheep placed in portable accumulation chambers), are still resource-heavy tasks and not suited to be used over weeks or months per study. Measurement of methane concentration from a breath subsample is constrained by the assumptions in converting a concentration to a flux and these are likely to be the downfall of any system that relies on spot samples without knowing the total output of the gas stream. So GEM has many positives in its favour that mean it is worth a very thorough test and improvement if possible.

This program has done that. We have:

- Conducted and submitted for publication 2 major validation studies of GEM as a tool to quantify DMP by cattle, finding no difference in MDP between GEM and RCs.

- Contributed advice to the manufacturers on modifications required to enable effective use of the GEM in remote locations
- Applied the GEMs in quantifying the impact of selection for feed efficiency (residual feed intake) on DMP of cattle in association with the cattle genetic project while grazing and in a feedlot.
- From this solid data set, we calculated the sampling regime (number of emission measurements) required to (a) quantify the mean emission rate of a herd by allowing for number of animals and (b) define the methane phenotype of an individual as may be required for genetic improvement.
- Led to development of the first GEM units for sheep in association with AgResearch.
- Validated sheep GEM units against RC emission measures.
- Applied cattle GEMS in multiple additional studies and environments in temperate and tropical areas.

Impact on practice and policy

The GEM is primarily a tool for measuring emissions rather than a means of reducing those emissions. As such it provides a means of verifying the efficacy of developing and approved mitigation methodology. The impact of this is that new methodologies for cattle can be verified on-farm to confirm that laboratory and animal-house research observed during development are being achieved on-farm. In so doing it provides a means to provide assurance that mitigation practices being considered as a means of delivering policy are effective in the in the commercial environment.

Contribution to GHG abatement

The GEM is not intended as an abatement tool, but it does have potential through delivery of 'medicated' supplements. We conducted a single study in which monensin together with a potential antiprotozoal were dispensed as additives in the supplement pellet (Section 3.3.6). In that study no difference in emissions from these additives was observed but the principle may well be applied and effective with other feed additives (such as nitrooxypropanol or essential oils)

Contribution to ERF methodology development

Methodology development is the principal application of the GEM. Having an assured accuracy (section 3.1) and an understanding of the number of samples that must be taken to obtain a precise estimate of the mean (section 3.4) provides a confidence to use the GEM for future emission measurement and abatement verification. It provides a tool for quantifying abatement in both research and commercial environments. In this program we used the GEM to quantify the effects of nitrate in a total mixed (feedlot) ration, as well as in liquid (molasses) supplements and in lick-blocks in rangeland environments. We also applied the GEM to assessing mitigation arising from selection of cattle for feed efficiency (RFI) and are currently applying the sheep GEM in measuring emissions from sheep rendered free of rumen protozoa, a potential methodology. In these things it has shown itself useful and has been developed well enough to provide robust measurement capability.

Contribution to productivity and sustainable land use

As a measurement tool, the GEM is not a vehicle for changing the productivity of animals except by inclusion of rumen modifiers in the pellets delivered. This application was outside the scope of the project but one study was made to prove in principle that this could be achieved.

Contribution to aim and expected outcome

This project was contracted to "*assess the current value of cattle GEM units for measuring daily methane output of cattle; will discover any impacts on grazing pattern of this measurement system, and will fund the development and evaluation of a GEM unit for sheep. GEM units will be applied in-field as a tool to verify at least 2 types of on-farm mitigation technology*".

All these aims have been met the project has delivered and/or demonstrated:

- A GEM cattle unit capable of operation in a remote location for at least 7days without human intervention.
- Optimised the construction and operation of GEM units for long term use in grazing environments
- GEM units for sheep available and accuracy assessed but showing some discrepancy in early comparisons
- An international protocol drafted for use of GEM units to verify emissions mitigation in sheep and cattle. This protocol using repeated measures of RC and GEM measures has led to 3 manuscripts being drafted for publication.
- Confirmation that the measurement process itself (using GEM units) does not cause changes in animal grazing behavior.
- Publications submitted documenting correlations between GEM and respiration chamber emission data, validating estimates of daily methane production from cattle GEM unit.

5. Future research needs

We have made one or two studies of all the critical factors associated with the cattle and sheep GEM units. There are a number of factors that warrant more or larger studies. In particular:

- Further validation of the sheep GEM in quantifying DMP
- Studies using larger paddocks and more animals to ascertain if the presence or use of the GEM unit affects grazing behaviour and nutrient intake of the animals being measured.
- Investigation of dispensing in-feed additives that could suppress emissions when delivered though the GEM feed dispensing mechanism, providing mitigation and measurement.

6. Publications

Velazco JI, Bremner G, De Barbieri I and Hegarty RS (2013). Short-term measurements to estimate methane emissions by beef cattle using the GreenFeen emissions monitoring unit. Recent Advances in Animal Nutrition – Australia 2013. (Ed. Cronje PB), pp.61-62. School of Environmental and Rural Science, University of New England, Armidale, Australia.

Velazco JI, Bremner G, Li L, Lujben K, Hegarty RS, Perdok H (2013b). Short-term measurements in beef feedlot cattle to demonstrate enteric methane mitigation from dietary nitrate. Advances in Animal Biosience 4(2), 579.

Hegarty, RS (2013). Use of short-term breath measures to estimate daily methane production by cattle. *Animal* 7, 401-408.

Velazco J.I.,Cottle DJ, Hegarty RS (2014). Methane emissions and feeding behavior of feedlot cattle supplemented with nitrate or urea. *Animal Production Science* 54, 1737-1740

Pickering N. K., V. H. Oddy, J. Basarab, K. Cammack, B. Hayes, R. S. Hegarty, J. Lassen, J. C. McEwan, S. Miller, C. S. Pinares-Patiño, Y. de Haas (2015) Genetic possibilities to reduce greenhouse gas emissions in ruminants. Animal (ANIMAL-14-10402R1; accepted on 02 Mar 2015.)

Velazco, J.I., D.G. Mayer, S. Zimmerman, R.S. Hegarty (2015a). Submitted to *Animal* (12/2014), accepted with revision, Resubmitted (2/2015).

Velazco J.I., D.G. Mayer, S. Zimmerman, R.S. Hegarty (2015). Use of short-term breath measures to estimate daily methane production by cattle. Submitted to Animal (12/2014), accepted with revision, resubmitted (2/2015).

Velazco JI, Herd RM, Cottle DJ, Hegarty RS (2015). Daily methane emissions and methane intensity of grazing beef cattle divergently selected for residual feed intake. Animal Production Science (submitted February/2015).

Cottle DJ, Velazco JI, Hegarty RS and Mayer DG (2015). Estimating daily methane production in individual cattle with irregular feed intake patterns from short-term methane emission measurements. Animal (submitted February/2015).

Cottle D.J., J.I. Velazco, D. Mayer and R.S. Hegarty (2015) How precise are enteric methane emission phenotypes or genotypes estimated from spot flux measurements? (submitted to Australian Association of Animal breeding and genetics for review).

Note: Roger Hegarty will joint author a paper on GEM at the 2016 Greenhouse Gases and Animal Agriculture conference in Melbourne Feb 2016.