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Novel individual enteric methane measuring system for multiple ruminants

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Abstract

Gas production in the rumen of livestock provides an important indication of metabolic function, as well as being a significant contributor to greenhouse gas emissions. The livestock industries thus require technology to measure enteric gas emissions from large numbers of individual animals simply, quickly, accurately and reliably. An intra-ruminal wireless sensor unit has been developed for measuring concentration of methane, carbon dioxide and hydrogen in the rumen, and is integrated with CSIRO’s wireless sensor network platform to provide telemetric capability. The complete unit includes miniaturized infra-red sensors, “Nano” circuit board, memory storage and battery supply, all of which are inserted into a diffusion cell that protects the device from the corrosive environment of the rumen. Once inside the diffusion cell, the sensors collect and transmit data from the equilibrated rumen gases to the outside world via radio transceiver. This will enable researchers and producers to develop, monitor and validate methane mitigation strategies to reduce emissions from grazing ruminants.

Executive Summary

Gas production in the rumen of livestock provides an important indication of metabolic function, as well as being a significant contributor to the anthropogenic greenhouse gas problem. The livestock industries thus require technology to measure enteric methane emissions from large numbers of individual animals simply, quickly, accurately and reliably to enable researchers and producers to develop, monitor and validate methane mitigation strategies to reduce emissions from grazing ruminants. Current technologies, such as respiration chambers and the sulphur hexachloride (SF6) tracer method, have limitations which make it difficult to reliably measure genetic and field variability and the effects of diverse farm management practices. As a result, there are currently no methods available to accurately and reliably measure methane production from large numbers of grazing animals. Therefore, a review of the literature was performed as part of the project to consolidate and update information on identifying novel methane measurement technologies from across diverse science and industrial sectors, which may have potential for application with large numbers of grazing ruminants. The review concluded that the idea of measuring rumen in grazing ruminants was feasible by using miniaturised infrared sensors, coupled with the technological advances made with wireless platforms.

Collaboration between CSIRO's Division of Livestock Industries and ICT Centre has developed an experimental wireless gas sensor node that uses miniaturized infra-red sensors for methane, carbon dioxide and hydrogen, and is integrated with CSIRO’s wireless sensor network platform to provide telemetric capability. The intra-ruminal wireless sensor unit includes the ICT Centre’s “Nano” circuit board with flash memory storage and its own battery supply, all of which is enclosed in a low density polyethylene (LDPE) plastic container with a 5% siloxane diffusion membrane that resides in the rumen of the animal. Once inside the diffusion cell, the sensors – protected from the corrosive environment of the rumen – collect and transmit data from the equilibrated rumen gases to the outside world via a 915 MHz radio transceiver. The system logs both methane and carbon dioxide concentrations in the rumen as well as temperature and battery voltage with a date and time stamp. The inbuilt flash drive logs data even when the animal is not in range of a base station for data acquisition and the stored data can later be downloaded at a time when the animal returns within range. The current radio range of the system is approximately 20 m using a laptop mounted antenna at the base-station unit and with the sensor device in the rumen of a large 850 kg steer. However, this can be extended to as far as 100 m by using a larger solar powered base-station antenna. Via

the radio transceiver in the sensor unit, it is possible to remotely vary the sampling time period from several minutes to several hours while the animal is at pasture.

The technology underwent significant improvements over the course of the project including (1) modifications to the outer membrane to increase diffusion rates and equilibration of gases, (2) infrared gas sensors that were resistant to corrosive gases such as H_2S, (3) the incorporation of activated carbon in the device as well as the design and manufacture of a cut-off valve to limit further exposure of sensors to the deleterious gases, (4) reduced power requirements. A joint provisional patent has been lodged covering the invention and intellectual property associated with the development of the device titled “System, method and device for measuring a gas in the stomach of a mammal”.

Ongoing animal research trials are being performed with sheep in respiration chambers to correlate daily emissions of expired methane with methane concentration measured intraruminally. Currently, the device is capable of detecting diurnal variations in methane and carbon dioxide concentrations in the rumen of experimental sheep in relation to feeding patterns. In addition, rises in temperature within the rumen due to fermentation can be monitored by the latest prototype of the intraruminal device. A shift in the ratio of carbon dioxide to methane gas levels has been detected with the intra-ruminal device in the same animal fed above and below maintenance rations. Measurement of hydrogen gas with the intra-ruminal device and the development of algorithms to predict methane emissions from gas concentrations in the rumen is still to be achieved but steady progress is being made in meeting these objectives in project B.CCH.1021. Additional time is required to develop relationships between intra-ruminal gas concentrations with actual emissions.

Final validation of the units will then allow large scale screening of animals for methane emissions to discriminate phenotypic differences (low *vs* high methane emitters) and effects of variation in feed base, climate and management. This technology is aimed at providing scientists and ultimately livestock producers with a tool to develop, monitor and validate methane mitigation strategies to reduce emissions from grazing ruminants.

Contents

1 Background

In Australia, ruminant livestock are the single largest source of methane emissions, accounting for nearly 71% of agricultural methane emissions to about 10% of Australia's net emissions of carbon dioxide equivalents (DCC, 2009). Methane is formed in the rumen when hydrogen, released by other microbes during fermentation of forage, is used by methane-producing archaea called methanogens to reduce carbon dioxide. Approximately 94% of the enteric methane emitted by ruminants is belched out and expired via the lungs (Murray *et al.* 1976). This loss of energy for the ruminant has been estimated to be between 2 and 14% of the animal's gross energy intake (Johnson and Johnson 1995; McAllister *et al*. 1996). A number of mitigation strategies for decreasing ruminant methane emissions from grazing animals have been suggested (Van Nevel and Demeyer 1995; Mbanzamihigo *et al*. 1996; McCrabb *et al*. 1997; Mathison *et al*. 1998; Hegarty 1999; Joblin 1999; Klieve and Hegarty 1999; Machmüller and Kreuzer 1999; Anderson *et al*. 2003; Machmüller *et al*. 2003a, 2003b; Wright *et al*. 2004), but assessing the effectiveness of many of these options depends on the accuracy of methane measurements.

Techniques for enteric methane emission measurements on individual animals can be classified into direct and indirect measurements. Direct measurement methods include total or partial enclosure of animals, whereas indirect methods include the use of tracers (isotopic and non-isotopic) and estimations based on rumen fermentation characteristics (Pinares-Patino and Clark, 2008). The use of respiration or methane chambers (direct measurement) and the sulphur-hexafluoride (SF6) tracer (indirect measurement) technique (Johnson *et al.* 1994a, 1994b) to measure methane emissions from ruminant livestock *in vivo*, are the two most widely used methods. In theory, the SF6 technique is suitable for grazing ruminants, but often when large numbers of animals are involved (n > 50), larger variability between and within animals on consecutive days have been recorded, especially when sheep have had little or no prior training with the apparatus. On the other hand, respiration chambers have greater sensitivity than tracer techniques, but they are not easily transportable, are usually limited to one animal at a time, and can be very expensive to construct.

The livestock industries thus require technology to measure enteric methane emissions from large numbers of individual animals simply, quickly, accurately and reliably to enable researchers and producers to develop, monitor and validate methane mitigation strategies to reduce emissions from grazing ruminants. Current technologies such as respiration chambers and the sulphur hexafluoride method (SF6) have limitations which make it difficult to reliably measure genetic and field variability and effects of diverse farm management practices. There are currently no methods available to accurately and reliably measure methane production from large numbers of grazing animals. Methane emissions data from respiration chambers do not take into account diet selection of grazing ruminants and day to day variation on total feed intake amongst animals.

The purpose of this project was to explore options using gas diffusion cells and electronic gas sensor technologies to identify and develop a robust method for measuring gas concentrations (methane and carbon dioxide) from individual ruminants with sufficient accuracy, low labour demand and simple data acquisition to allow large scale screening of animals for methane emissions to discriminate phenotypic differences and effects of variation in feedbase, climate and management.. The project has therefore developed a novel intra-ruminal gas measuring device utilizing the latest electronics to estimate greenhouse gas (GHG) concentrations at the source of production in the rumen. Miniaturization of electronic components in conjunction with CSIRO's 'Nano' technology, improvements in battery power utilization and the development of sensor networks have made the option of deploying an intra-ruminal gas measuring system a real possibility.

The project links to the Climate Change Research Program (CCRP) which has an objective to develop commercially viable tools and management techniques for farmers, foresters and fishers to manage emissions and adapt to climate change. The major objective of this national collaborative program of research on methane emissions from ruminants is to deliver knowledge and technologies to enable producers to breed and/or manage ruminants to significantly reduce methane emissions while maintaining livestock productivity for a viable agricultural industry. Through effective integration and management of research effort this program will:

- support the development of methane abatement technologies
- promote development of farming systems with low net emissions
- provide data for continued improvement in comprehensive land systems greenhouse gas accounting
- provide information for informed decisions relating to agriculture and the Carbon Pollution Reduction Scheme and the positioning of the Australian livestock industry globally

2 Project Objectives

1. By May, 2009 complete a knowledge and opportunity audit of novel methane measurement technologies which have the potential to be developed and adapted for use to measure methane emissions from large numbers of grazing ruminants.

2. By April, 2010 provide data demonstrating the value and requirements of integrated rumen gas measures and profiles in relation to predicting greenhouse gas production by individual animals, and which provides all the information needed to construct a measurement and data collection system suitable for use with grazing cattle.

3. By December, 2011 develop a practical, reliable, repeatable individual ruminant methane measurement and data collection system suitable for use with large numbers of grazing animals and sufficiently precise to discriminate natural phenotypic variability in methane emissions and responses to mitigation strategies. Upon successful completion of this objective, participation in further research work on evaluating heritability of animal emission diversity and interactions with grazing systems management will be considered.

3 Methodology

3.1 Knowledge and Opportunity Audit

A knowledge and opportunity audit of the literature for novel methane measurement technologies that have potential to be developed and adapted for use to measure methane emissions from large numbers of grazing ruminants was performed. The audit was reviewed and accepted by a small panel of experts. The report recommended that commercially available (e.g. Dynament, City Technology) infrared sensors be used to measure methane, carbon dioxide, and hydrogen. These sensors can be integrated with the CSIRO FLECK™ platform with telemetric capability and the sensors powered with its own 9V battery supply. The complete unit (sensors, FLECKTM, and battery supply) will be small enough to fit inside an intra-ruminal diffusion cell which will reside inside the rumen and be protected from the corrosive environment with a gas diffusible membrane. The audit also recognised that the commercialisation of biosensor technology has lagged behind the output of research laboratories. Although many biosensor-related patents are filed each year, very few play a prominent role in clinical diagnostic, food industry, environmental agricultural or veterinary applications. There have been many reasons for the slow technology transfer from the research laboratories to the marketplace: cost considerations, stability and sensitivity issues, quality assurance, and instrumentation design. Many of these barriers are technical, and are being addressed as the technology advances. It is envisaged that CSIRO would drive the commercial process through commercial partners. The audit also identified that the potential applications of biosensors in animal agriculture are numerous and that there was a marked increase in scientific activity in relation to these applications.

3.2 Development of the Technology

3.2.1 Early Dynament® Gas Sensor Prototypes

In search of a method to quickly and reliably measure methane emissions from large numbers of individual animals, researchers from CSIRO's Division of Livestock Industries initially developed an early prototype device that was capable of measuring carbon dioxide and methane concentration data from a fistulated steer. Initially, 20mm diameter infrared sensors for methane and carbon dioxide were placed in liquid sealed polyethylene cylinders with cables (35cm long) that extended from the rumen of a steer via the fistula to the outside of the animal as shown in Figure 1.

Figure 1. Early intra-ruminal device incorporating 2 gas sensors housed in a sealed low density polyethylene Nalgene® container. Gas diffusion across the plastic proved to be slow with a long lag time to reach equilibrium. The system was very labour intensive as fistulated animals had to be yarded to collect rumen gas concentrations in real-time.

This allows the sensor cables to be plugged into a configuration unit which powers up the sensors and following a brief auto-calibration the percentage of gas present in the gas diffusion cell can be determined using a laptop computer to capture the data. Gas diffusion across the low density polyethylene plastic vessel housing the sensors showed equilibrium was established over several days. The sensors were calibrated in controlled laboratory studies with known concentrations of gases and then evaluated in animals in the field. Field testing of early prototype sensor devices took place in fistulated steers because they could accommodate the larger and bulkier units prior to miniaturisation of the devices. Reduction in size of the units was paramount so that they could be inserted through a 50mm wide opening in a sheep rumen cannula.

Due to the limitations of data acquisition and transmission of the initial device, research staff enlisted the help of the CSIRO's Information and Communication Technologies (ICT) Centre to develop a more sophisticated prototype. The result of this collaboration resulted in an experimental wireless gas sensor node that uses miniaturized infra-red Dynament® gas sensors for methane and carbon dioxide and is integrated with CSIRO's wireless sensor network platform to provide telemetric capability.

3.2.2 Initial Wireless Intra-Rumen Sensor Unit

The intra-rumen wireless sensor unit, shown in figure 2, is based on the Nano circuit board and was designed and developed by the CSIRO ICT Centre Engineering Team. The miniaturized infra-red Dynament® gas sensors for methane and carbon dioxide are mounted on both ends of the gas sensor node.

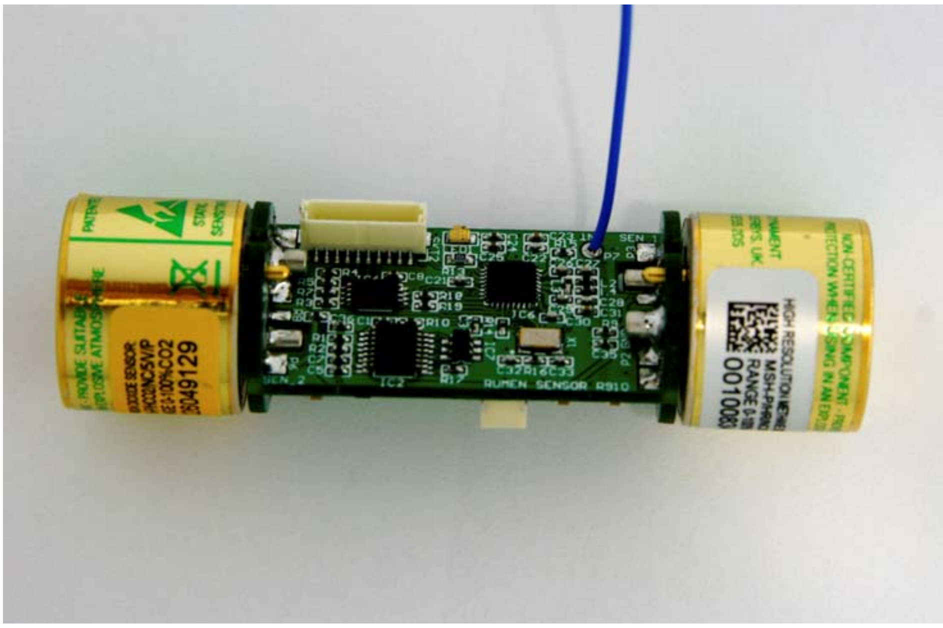

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Figure 2. Initial wireless Intra-rumen sensor unit

The intra-rumen wireless sensor has a number of key features and specifications including:

- Two sockets for Dynament® Infra-red gas sensors to measuring 0 to 100% (0.1%) concentrations of methane and carbon dioxide gases.
- Nordic 8051 microcontroller and 915 MHz radio transceiver
- 16-Bit, 4-Channel Serial Output Sampling Analog-To-Digital Converter with precision voltage reference
- Temperature and battery voltage sensing
- 1 MByte serial-interface data Flash memory
- Two switchable regulators for the gas sensor power
- 916 MHz quarter wave wire antenna
- Radio range 30 to 100 m inside rumen and 750 m line of sight
- Size, including two gas sensors and excluding batteries, is 80 mm long x 20 mm round
- External battery power is 3.5 to 6 Volts @ 200 mA maximum current

The schematic and printed circuit board (PCB) is designed using small components including 0402 sized surface mount capacitors and resistors. A conformal coating is applied to protect the electronics against moisture, chemicals, and temperature extremes inside the harsh environment of the rumen.

Figure 3 shows the intra-rumen wireless sensor, rechargeable NiMH batteries and the low density polyethylene plastic container used in the initial experiments. To reduce size rechargeable AAA @ 1.1A hour batteries have been used in the sheep sensor device. Larger rechargeable AA @ 2.4A hour batteries and a larger container were used for the cattle sensor device to increase battery life.

Figure 3. Intra-rumen sensor unit, batteries and polyethylene plastic container

The software developed for the Intra-rumen device is a cut down and optimised version of the Fleck Operating System (FOS). Key components of FOS were modified for use with the intra-rumen device allowing it to maintain radio compatibility to with a Fleck device.

Two of these key software components were the tagged data format (TDF) and the remote procedure calls (RPC). Utilising the TDF provided a standard to base the data transmission on, and to easily interface the sensor data stream with visualisations and data logging via the database backend. Preserving RPC as part of the software for the intra-rumen device provides the ability to issue remote commands which can be executed on the device.

The current software implements a full medium access control layer, and a multi-hop routing layer for radio communication, and logs methane, carbon dioxide and temperature sensor data to an on-board flash chip. Using the RPC technology, sensors can be sampled on demand, sample periods can be modified, and the state of the program can be varied, including a special over the radio download mode for downloading the sensor data stored on the flash chip. A PC based python software utility has also been developed to handle the PC communications with the intra-rumen device and to store the data after a download.

The radio telemetry system used on the intra-rumen sensor device (Figure 4), has been designed and developed to allow transmission of data from grazing animals in the field to a laptop computer via a USB mounted antenna. This system is undergoing rigorous testing in the field in both fistulated sheep and cattle. The gas sensor device has both a radio transmitter as well as a receiver. This allows the operator to make changes to the sampling mode remotely whilst the animal is still at pasture without the need to yard the experimental animal. Data can then be downloaded at a suitable time. Changing the mode on the software application allows real time data acquisition to be accessed in the field as well as the ability to download all stored data at a later date over the course of an experiment.

Figure 4. Intra-rumen sensor unit radio telemetry system installed on a laptop

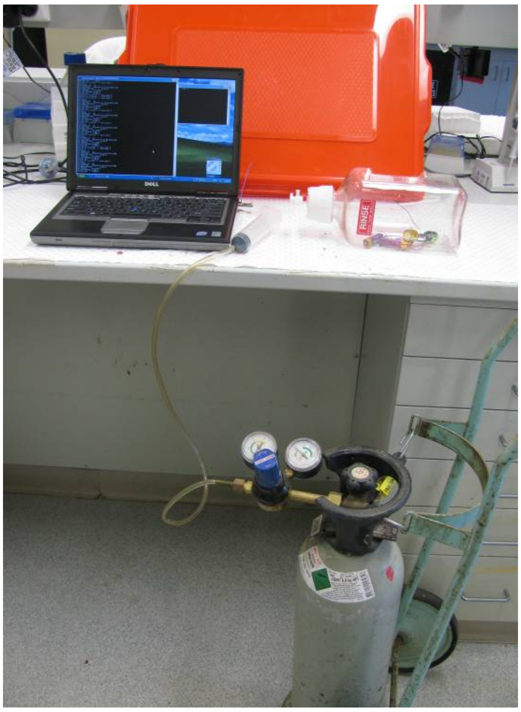

Figure 5. Calibration tests of the gas sensors under laboratory conditions using intra-rumen wireless telemetry.

A method to test the calibration of the infra-red Dynament® methane and carbon dioxide gas sensors is show in figure 5. Both gas sensors are tested at zero and span gas levels within a glass vessel. The results are recorded via the intra-rumen sensors wireless telemetry.

A number of problems where eventually located with initial wireless intra-rumen sensor unit after undergoing rigorous testing in the rumen of experimental animals and these are outlined later within the report. This has lead to the development of completely new intra-rumen sensor unit platform using new hardware and software, new battery technology and new more permeable containers.

3.2.3 Development of the Diffusion Cell

The results from utilising the initial wireless intra-rumen sensor in the polyethylene plastic container confirm there is a poor response time to changes in the gas concentrations within the rumen resulting from the low permeability of the container for methane and carbon dioxide gases. The project team therefore needed to locate a much more permeable material for the diffusion cell part of the container.

The project team examined many different plastics for their ability to allow rapid diffusion of methane and carbon dioxide which would be far superior to the original low density polyethylene Nalgene® container. Following consultation with researchers at CSIRO’s Materials Science and Engineering Unit, several possible candidate materials were identified. This was narrowed to a couple of suitable membranes, one of which has been successfully tested in diffusion cells to measure O_2 in soil profiles undergoing rehabilitation following petrochemical contamination. Thin membranes constructed from polydimethyl siloxane (0.2mm) were considered suitable for withstanding the rigours of the rumen environment and maintaining their integrity whilst allowing gas to diffuse in both directions.

The polydimethyl siloxane membrane has been mounted on an empty cattle controlled release device (CRD) capsule, with approximately sixteen, 10mm diameter holes pre-drilled into the barrel of the unit. The CRD has dimensions of 160mm length x 38mm diameter, with 85mm wings that fold flat against the barrel of the unit when dosing to the animal, and then flare out upon reaching the rumen. This change in the geometry of the CRD overcomes regurgitation of the capsule by the animal during times of rumination. In order to adequately seal the unit and prevent liquids moving inside the capsule, the membrane ends have been overlapped and joined with a silicone adhesive. The barrel of the CRD has been rebated at each end to allow a compression ring to be seated in conjunction with the adhesive. This effectively blocked rumen fluids from gaining access to the electronics inside the device.

The open end of the CRD has a specifically designed nylon cap to seal the unit after the electronics have been placed inside. The nylon end-cap is rebated with a groove to allow an “o” ring to be seated in the groove and provide a good moisture barrier to the electronics inside as shown in figure 6.

Figure 6. Diffusion cell made from a controlled release device (CRD) and a polydimethyl siloxane membrane

Testing of the new diffusion cell and the initial wireless intra-rumen sensor unit in cattle and sheep concluded that the response time to changes in gas concentrations within the rumen were significantly improved. The increased concentration of other gases, such as hydrogen sulphide, however, now caused contamination of the infra-red Dynament® methane and carbon dioxide gas sensors. The resulting permanent damage to the gases sensor's internal surface caused incorrect gas sensor readings.

3.2.4 Overcoming Infra-Red Gas Sensor Contamination

The new diffusion cell, shown in figure 6, used to increase the response time of methane and carbon dioxide concentrations within the rumen, presumably also increased concentration of other gases, such as hydrogen sulphide. This has caused corrosion of the infra-red Dynament® methane and carbon dioxide gas sensors. The resulting permanent damage to the gas sensor internal surface is shown in figure 7.

Figure 7. Infra-red Dynament® methane gas sensor contamination

After a period of 3 to 4 days deployment of the initial intra-rumen wireless sensor device in the rumen, there was a zero calibration drift from the Dynament® gas sensors. Upon close inspection at our laboratory and further examination at the laboratory of the manufacturer, the problem was identified as corrosion on the reflective surfaces of the infrared sensor housing. The most probable cause for the corrosion was the presence of hydrogen sulphide crossing the membrane from the rumen, in a warm moist environment.

To solve the gas sensor contamination problem a worldwide search for a more robust gas sensor was initiated. The search identified the e2v® infra-red gas sensor, as the best gas sensors for the initial intra-rumen wireless sensor device, as it is manufactured from stainless steel and has a double coating of gold plate on the internal reflective surfaces. The inert properties of the construction materials of the e2v® sensor make it more suitable for the harsh environment within the rumen.

There are a number of advantages of the e2v® infra-red gas sensor including:

- Dual carbon dioxide and methane sensor in the one unit
- 100% concentration range for both methane and carbon dioxide gases
- Much lower power consumption due to a single infra-red lamp, shorter sample time and more efficient electronics
- Stainless steel and double coating of gold plate on the internal reflective surfaces
- Much lower sensor cost and smaller size as only one is required

There are also a number of disadvantages of the e2v® infra-red gas sensor including:

- Over 100 extra electronic components need to be fitted to the small intra-rumen wireless sensor device circuit board
- Much more complex signal processing software needs to be written for the gas sensor.

Figure 8 shows the evaluation circuit board with the capability of measuring both methane and carbon dioxide, at 100% range, using the one sensor unit. Almost all of the extra components on the printed circuit board need to fit on the new intra-rumen wireless sensor device which will be approximately one-tenth of the size. The smallest components will need to be used, for example 0201 size surface mount resistors and capacitors. Some optimisation of the circuit will also need to be made.

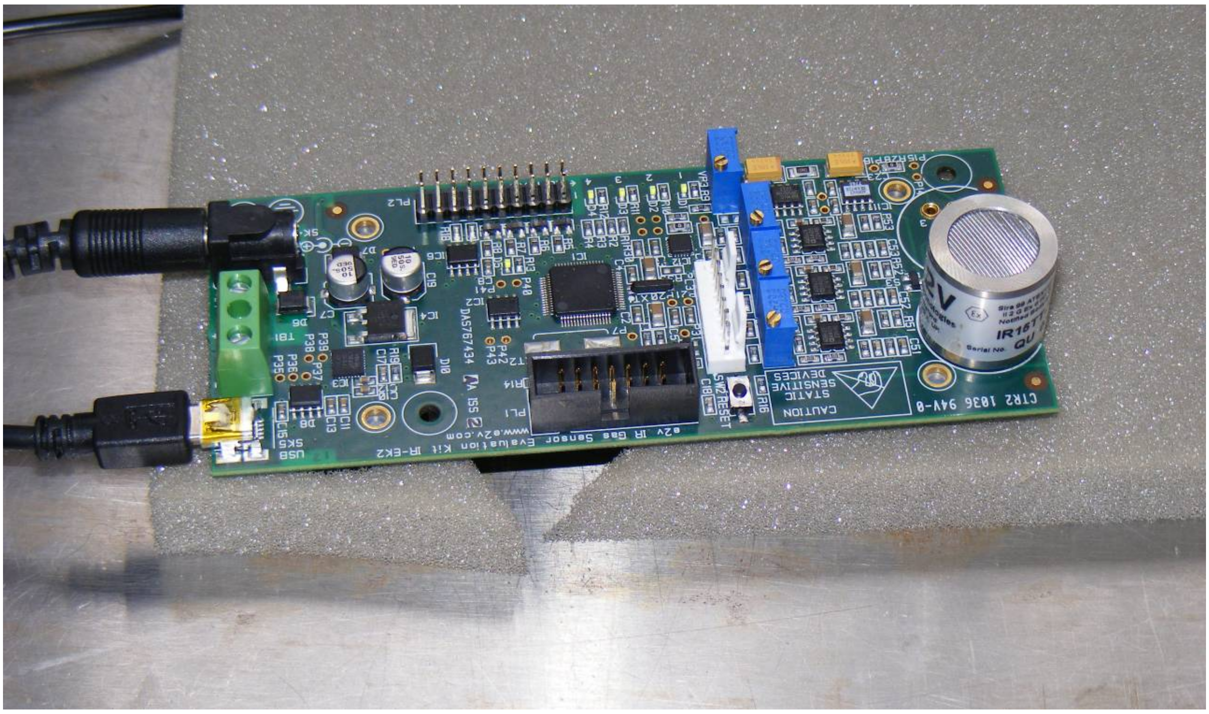

Figure 8. Dual E2v® gas sensor (RHS) mounted onto an evaluation circuit board with the capability of measuring both CO_2 and CH_4 from the one sensor unit.

The Dual E2v® gas sensor mounted on the circuit board and placed inside the diffusion cell within the rumen was evaluated. However the photodiodes were also contaminated ands showed corrosion and thus aA second solution was required to help solve the remaining contamination problem (figure 9). To help solve the contamination problem a reduction in the water vapour and hydrogen sulphide on the sensor had to be made. A search identified an industrial solution, specialised grade pelletised activated carbon, Acticarb EA1000K, from Activated Carbon Technologies Pty Ltd which is primarily used to remove hydrogen sulphide from sewage plants. A small amount of activated carbon, 20g, is incorporated in a satchel within the CRD to eliminate the damage to the gas sensors. The material however can saturate and has a finite life. The proposed solution to the longevity of the activated carbon and the gas sensor is a small cut-off valve which is controlled by a very small electric motor (Figure 10). The valve will be closed when a gas reading is not required. On the gas sensor side of the valve there will be a satchel of pelletised activated carbon which will reduce water vapour and hydrogen sulphide. When a gas reading is required the valve will open and then close again when complete. This will minimise the exposure of the activated carbon to water vapour and hydrogen sulphide and prolong the life of the activated carbon and the gas sensor.

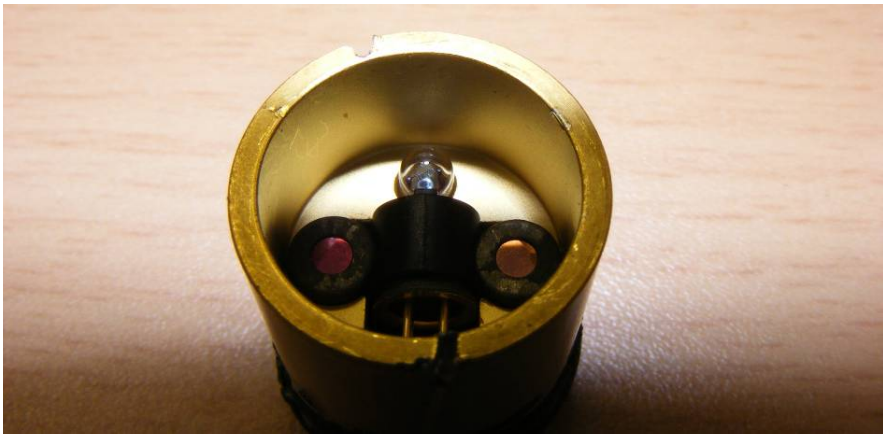

Figure 9. Dual E2v® gas sensor with the top removed showing small contamination on the photodiodes after it was placed inside the diffusion cell and installed in a rumen.

Figure 10. Gas cut-off valve with small electric motor

3.2.5 Evaluating the e2v® Infra-Red Gas Sensors in the Rumen

Initial tests were conducted using unpowered e2v® gas sensors in the rumen with 18g satchels of activated carbon, Acticarb EA1000K, which is used to take up hydrogen sulphide and moisture. From these results the project team developed a wired system for sheep using the e2v® gas evaluation circuit board shown in figure 11.

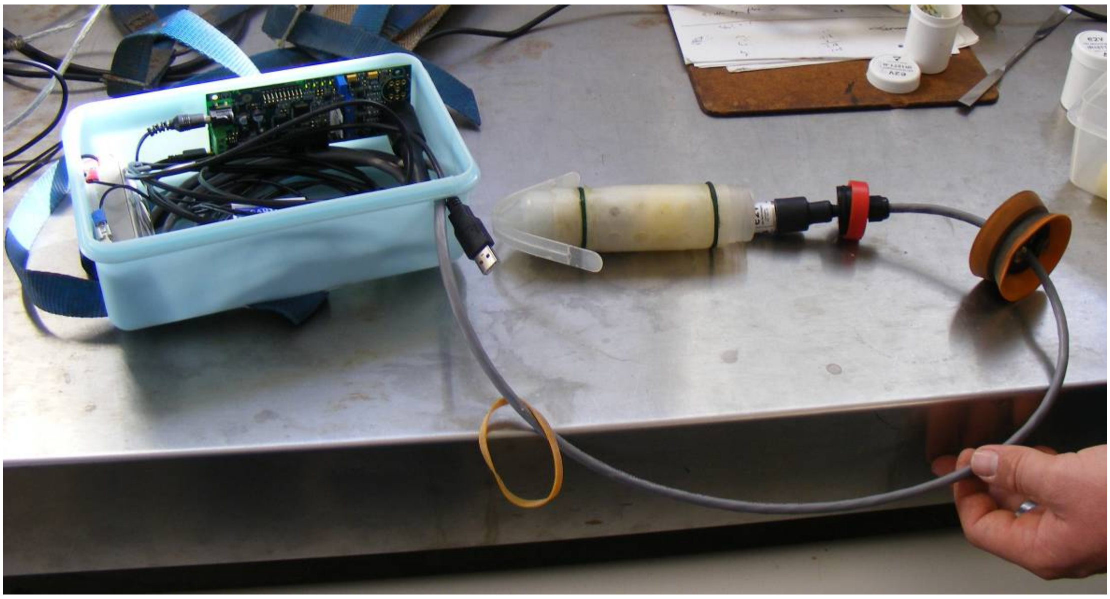

Figure 11. e2v® intra-rumen gas testing assembly

The e2v® gas testing assembly was mounted in a rumen fistulated sheep as shown in Figure 12 and a laptop was connected, to periodically record the methane and carbon dioxide gas concentrations.

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Figure 12. e2v® intra-rumen gas testing assembly on a rumen fistulated sheep

From the intra-rumen e2v® gas testing results a new wireless intra-rumens sensing unit was designed and developed by the CSIRO ICT Centre Engineering Team, with many improvements over the initial wireless intra-rumens sensing device.

3.2.6 Final Wireless Intra-Rumen Sensing Device

3.2.6.1 PACP Serqet Hardware Development

After solving the infra-red gas sensor contamination problems a new wireless intra-rumen sensor unit needed to be designed. The new intra-rumen wireless sensor unit, shown in Figure 13, is based on the PACP circuit board and was designed and developed by the CSIRO ICT Centre Engineering Team. The miniaturized infra-red e2v® dual gas sensor for methane and carbon dioxide is mounted on one end of the gas sensor node.

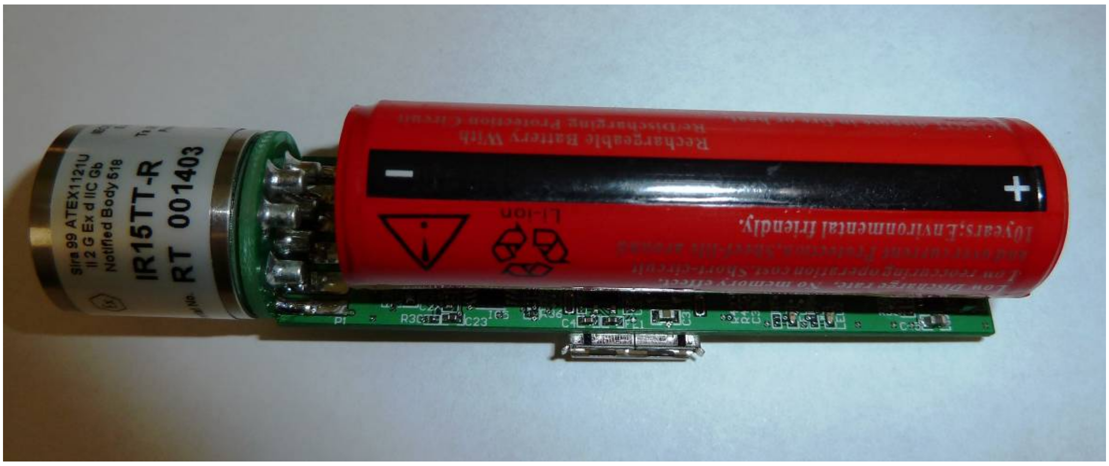


Figure 13. PACP Serqet wireless Intra-rumen sensor unit

The new intra-rumen wireless sensor has a number of new key features and specifications including:

- Socket for one miniaturized infra-red e2v® dual gas sensor to measure 0 to 100% concentrations of methane and carbon dioxide gases.
- Texas Instruments CC430F5137 microcontroller and 916 MHz radio transceiver
- 16-Bit, 8-Channel Serial Output Sampling Analog-To-Digital Converter with precision voltage reference
- Pressure and air temperature sensing
- Very low powered gas sensor filter circuits for the reference, methane and carbon dioxide signals
- Gas sensor thermistor for gas sensor temperature monitoring
- Battery voltage, charging voltage and current monitoring
- 1 MByte serial-interface data Flash memory
- Switchable regulators for the gas sensor and gas circuit power
- Efficient 5 volt step-up converter to increase gas sensor lamp voltage and gas sensitivity readings
- Efficient on-board Li-ion battery charger which can be used for energy harvesting
- Bidirectional DC motor driver to control the gas cut-off valve
- 916 MHz quarter wave wire antenna
- Improved radio range from inside the rumen and up to 3 Km line of sight
- Size, including two gas sensors and battery, is 100 mm long x 20 mm round
- Board mounted rechargeable 3.6 volt @ 3A hour Li-ion battery

The schematic and printed circuit board (PCB) is designed using the very small components including 0201 sized surfaced mount capacitors and resistors. A conformal coating is applied to protect the electronics against moisture, chemicals, and temperature extremes inside the harsh environment of the rumen.

3.2.6.2 PACP Serqet Software Development

The software used in the intra-rumen device, the Serqet platform, is a variant specific to this application within the family of Pervasive Autonomous Computing Platform (PACP) devices, designed specifically for intra-rumen sensing within livestock. Common to all devices of this class are the main microcontroller and radio, specifically the Texas Instruments CC430F5137 system-on-chip. This small footprint chip provides a 16-bit ultra-low-power MCU (up to 20MHz), 32KB flash, 4KB RAM, the CC1101 (digital) radio with AES-128 encryption and a 12-bit ADC, making it an ideal starting point for a small-sized wireless sensing platform. This does, however, introduce significant software constraints that must be considered when designing the system logic. The Contiki OS, developed by the Swedish Institute of Computer Science, SICS, is a small-footprint, generic and extensible operating system specifically designed for resource constrained micro-controllers. Development of the OS continues within the open-source community and can be used unencumbered for commercial applications. We have adopted the Contiki OS as the primary OS for the PACP family of devices, with the development of fundamental contiki drivers for the MCU largely pioneered by the Serqet platform.

The intra-rumen software design has 3 significant aspects:

- Core (cross-platform) Drivers
- Platform-specific Sensor and Peripheral Drivers
- Application Logic

3.2.6.2.1 Core Software Drivers

Communications

Sensors for the Serqet platform communicate via 3 mechanisms; Serial Peripheral Interface (SPI); Inter-Integrated Circuit (I2C), also known as "two-wire interface"; and via direct interface to the MCU via the Analog-to-Digital-Conversion (ADC) and Input/Output (I/O) pins. The CC430F5137 shares common lines for the SPI, I2C and serial (RS232) communications, and platforms would typically be limited to supporting either SPI or I2C, but not both. A number of sensors, which are needed for intra-rumen sensing, support only one communications interface. Therefore switching circuitry is needed to enable support for both SPI and I2C. This requires additional software development, as an SPI abstraction within the Contiki OS, to support this useful feature. The I2C interface exists within Contiki, however a small addition is also required to handle the switching from SPI to I2C and vice versa. To keep complexity to a minimum, one of the two communications buses is dedicated to serial communications (RS232) while the other handles SPI/I2C. Contiki readily provides high-level abstraction to the RS232 logic, enabling debugging information to be sent out via the serial interface, however the lower RS232 layer also needs to be interfaced in order to receive commands and send out sensor data during the serial download process. Further details of this process are provided in the Data Download section. The RS232 is also used for reprogramming the node. But it’s reprogramming is not robust, and often requires many attempts before the code is successfully loaded. Investigation revealed the timing of the uploading program was not optimal. Following optimisation of this utility, code-loading is now robust and substantially faster.

LEDs, ADCs, General/O

Contiki includes a number of general abstractions for setting and toggling pins of the MCU. These have been utilised to provide; LED functionality, primarily used for debugging and visual inspection of operation, and the setting of logic levels required by various sensors. The ADC functionality of the CC430F5137 is reasonably rich and hence quite complex. Contiki currently does not provide open-source code for the CC430F5137 chip and so ADC logic has been developed so that the battery voltage, charge voltage and charge current can be measured accurately. This provides valuable information on the actual energy dissipation during experiments.

Real-Time Clock

The Real-Time Clock (RTC) is a low-power timing mechanism provided by the CC430F5137. Currently only periodic timing (non-changeable) is supported on the current device, however general arbitrary timing is under development. The period is currently fixed at 933 seconds (15 minute interval + 33 seconds sample time) which is twice the sample period required for the current experiments (~ 30 minutes). A software bug currently exists where occasionally the RTC generates an erroneous value when read, however it does not appear to affect the period of the sampling. As a sample sequence number is stored with each measurement and the "time-stamp" of the measurement can be corrected post-download.

3.2.6.2.2 Platform-specific Sensor and Peripheral Drivers

Flash Driver

The AT45DB flash chip uses the SPI for communication. Existing drivers have been modified to support the Serqet platform using Contiki. The flash chip provides 4,096 pages (256/264 bytes/page) of storage. Due to various specifications, it is preferable to complete full page writes to ensure the data is safely stored on this non-volatile memory. Due to the small amount of data written each sample period we currently write one reading per page. When the sample rate of the sensors increases, this will ultimately be changed to utilise the memory more efficiently. At present we are investigating the Coffee File System (CFS), which is native to the Contiki OS, as an efficient storage mechanism.

3.2.6.2.3 Dual-Gas Sensing

The e2v® IR15TT-R dual gas sensor is capable of sensing concentration of methane (CH_4) and carbon dioxide (CO_2) gases. The circuit and logic required to drive and obtain readings is relatively complex to most sensors. First the heat lamp of the sensor must be turned on for a period of up to 30 seconds before valid readings commence. This requires both IO and timing logic. Secondly, a 4Hz pulse (square wave) must be generated into the sensor. The generated outputs of the reference, CH_4 and CO_2 signals are sinusoidal and the peak-to-peak voltages of these 3 signals is obtained by sampling each numerous times (via ADCs) and finding the difference between the minimum and maximum values. Upon successful sampling of the sensor, the circuitry to the sensor is switched off to save power.

3.2.6.2.4 Pressure and Temperature

The Bosch BMP085 pressure sensor is interfaced via I2C. The sensor is also capable of accurately measuring temperature (to within +/- 0.5C) which can be used for more accurate conversion of the pressure reading. Uncompensated temperature and pressure readings are sampled and converted to compensated temperature and pressure readings via very complex formulae.

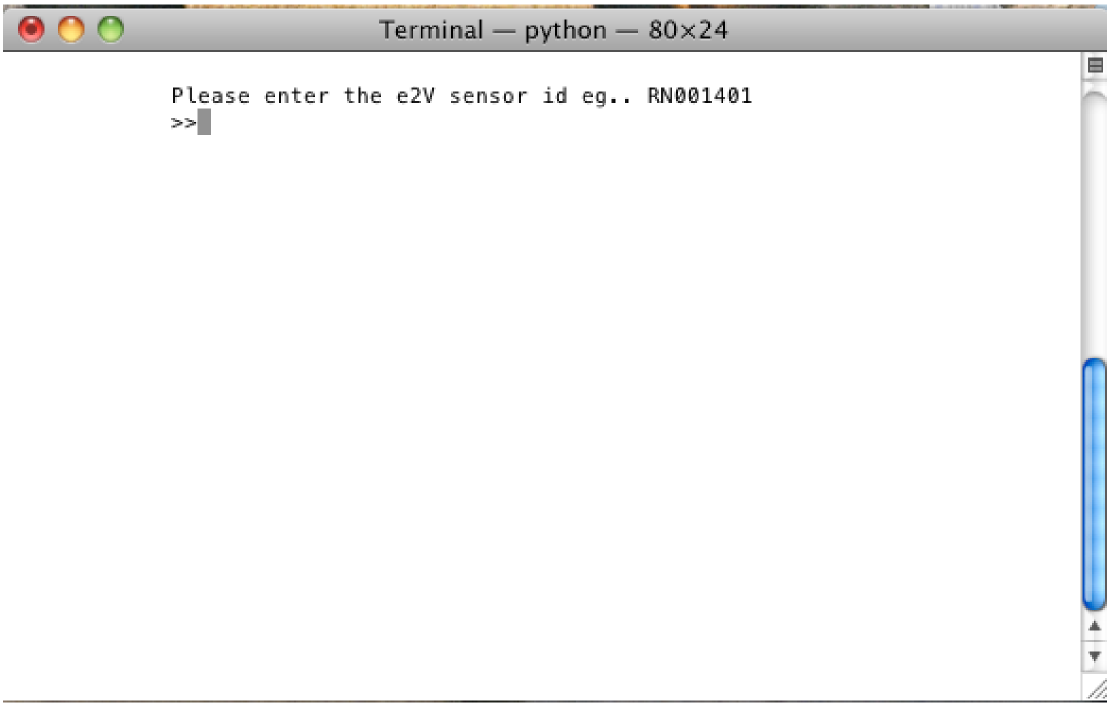
3.2.6.2.5 Application Logic

The application logic is comprised by two threads (separable logic) and the initialisation logic. The "Sample Sensor" thread is responsible for periodically activating the sensors, reading their values, and storing this to flash. As the device may be restarted for multiple experiments between downloads, the application first scans the flash memory for a free area to begin writing data. This thread maintains a counter (initialised at 0) for each sensor reading and stores that counter with each flash write of all the sampled sensors. This has proved useful for correcting some erroneous readings of the sample time, measured by the RTC, which is also saved with the sensor data.

The "Download Manager" thread provides a method for transferring the saved sensor readings from the Serqet node to a PC. It waits for incoming commands via the serial port and handles; reading of data from flash; packetizing the data, and finally sending that data back across the serial port to the PC. The PC interface for this process is discussed in the following section.

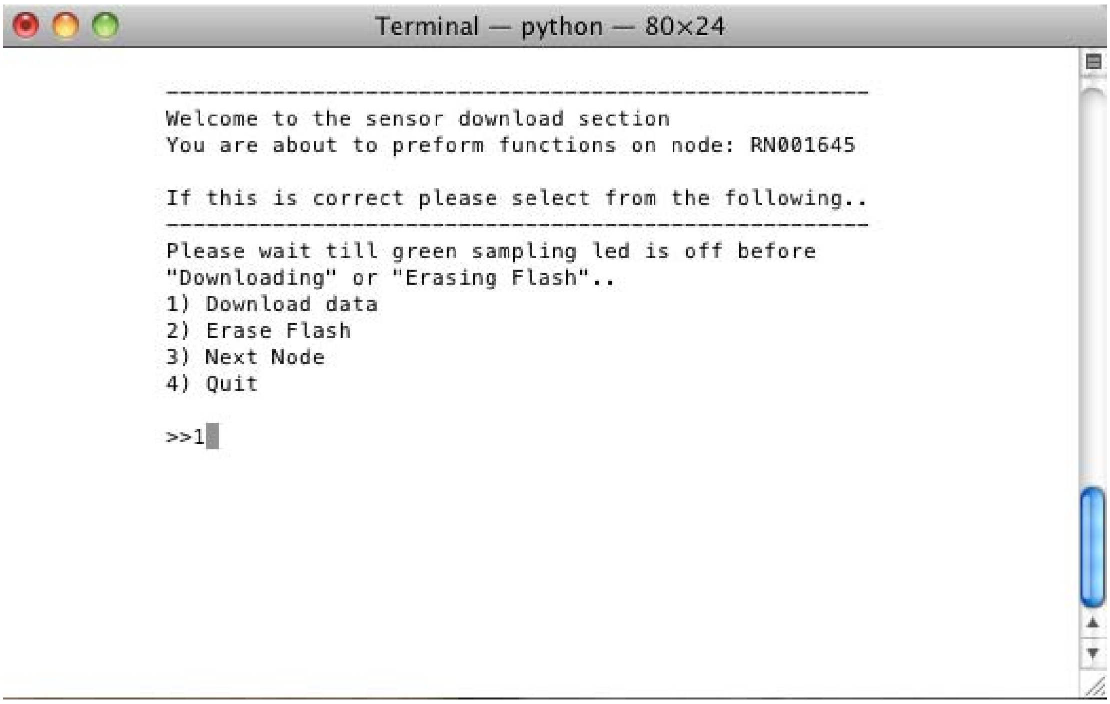
3.2.6.3 Download Manager PC Client

The download interface for the PC has been written in a cross-platform language, Python, so that it can work on Windows, Apple (OS X) and Linux. The text-based interface, shown in Figure 14 and 15, allows the user to download data into a comma separated values (CSV) compatible format which is readily imported into applications such as Microsoft Excel. Typically, however, once the data is downloaded, a parsing script is run which converts the raw sensor readings into conventional units, before it is imported into Excel. The interface is also used to clear the contents of the flash memory and further commands, such as setting the sample period, will be introduced in future. Ultimately the interface will be web-based and will communicate wireless with the Serqet nodes.



```
Please enter the e2V sensor id eg.. RN001401
>>
```

Figure 14. Download Manager PC Client Initial Screen



```
-----------------------------------------------------
Welcome to the sensor download section
You are about to preform functions on node: RN001645

If this is correct please select from the following..
-----------------------------------------------------
Please wait till green sampling led is off before
"Downloading" or "Erasing Flash"..
1) Download data
2) Erase Flash
3) Next Node
4) Quit

>>1
```

Figure 15. Download Manager PC Client Menu Screen

3.2.6.4 Software Testing and Calibration

A significant period has been spent comparing readings sampled by the Serqet node against those sampled by the e2v® Infrared Gas Sensor Evaluation Kit (IR-EK2) using the same sensor. The manufacture's coefficients match those readings sampled with the Serqet node within acceptable tolerances, thus providing an assurance that the sensor readings by the Serqet node are accurate as those sampled using the manufacture's hardware.

The experiments were planned to run uninterrupted for up to 5 days. The current logic consumes less than 6mA and with the 3A hour battery will operate for approximately 20 days. Before the intra-rumen experiments, 10 units have been tested over a 3 day period to ensure stability of the code.

3.2.6.5 Future Development

Ultimately, commands and data will be communicated wirelessly via the digital radio. Drivers for single-hop communications have been developed and tested, while drivers for robust multi-hop communications are still under development. It is envisaged that data and commands will communicate from the intra-rumen unit to a nearby device (likely to be co-located on the animal), then through multiple hops via stationary, permanently deployed nodes, to a base node. The permanent base node will upload data to a database where it can be visualised or downloaded via a web-interface. This web-interface will also facilitate wireless commands to the devices such as dynamically setting the sample period during experimentation.

Reducing power consumption is always an ambition with finite energy devices and ongoing investigation of methods for achieving lower energy consumption. The core drivers draw current continuously at approximately 4 to 5mA and so this will be the immediate future focus. As the limits of potential savings are reached on this, attention will turn to the peripherals and sensors, in particular the gas sensors which draw significant current. Determining the minimum time the sensors need to be active in order to achieve a reliable reading will be essential to minimising power consumption and increasing the longevity of the devices.

Sensor poisoning is still a concern despite the promising experimental results with activated carbon. In order to reduce the exposure to deleterious substances, namely hydrogen sulphide (H_2S), a cut-off valve has been developed which will in essence "duty-cycle" the exposure of the gas sensor. A hardware prototype has been developed and the logic to drive this will be part of future tests.

Observations from the previous and current trials show significant changes in gas concentrations, pressure and temperature, can occur rapidly in between long periods of relatively constant sensor readings. To provide richer data sets, the sampling of sensors will be non-aligned so that each sensor can be sampled at individual sample rates. Furthermore, the sampling may be dynamic (non-periodic) based on the differential sensor readings, i.e. sampling at higher frequency rates when the readings are more dynamic. This will offer a balance between conserving power and providing higher resolution sensor data.

3.2.7 Oxymax Calorimeter System and Respiration Chambers

The Oxymax calorimeter system from Columbus Instruments (Ohio, USA) is shown in Figure 16. The system is connected to respiration chambers at F D McMaster Laboratory, as shown in Figure 17, and will provide measurements of exhaled methane and carbon dioxide gases from up to six fistulated sheep .

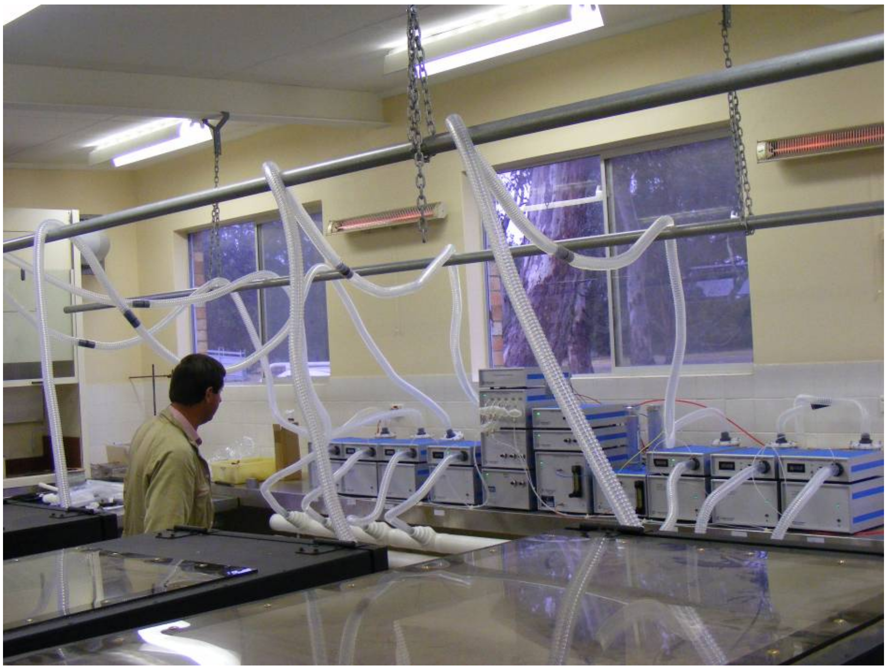


Figure 16. Oxymax Calorimeter System and associated respiration chambers at F D McMaster Laboratory.

The Oxymax Calorimeter system was commissioned in conjunction with purpose built sheep respiration chambers. Testing and optimizing of the system is providing valuable data on the capability of the system to detect variations in feed intake and animal to animal variation.

Figure 17. Open respiration chambers at F D McMaster Laboratory

3.3 Experimental Testing Procedures

3.3.1 Early Dynament® Gas Sensor Intra-Rumen Measurements

Dynament® infra-red gas sensors for methane and carbon dioxide are placed in liquid sealed polyethylene cylinders with cables (35cm long) that extend from the rumen of a steer via the fistula to the outside of the animal. This allows the sensor cables to be plugged into a configuration unit which powers up the sensors and following a brief auto-calibration the percentage of gas present in the gas diffusion cell can be determined using a laptop computer to capture the data. Figure 1 shows the Dynament® infra-red gas sensors and the cable used for the experiment.

3.3.2 Initial Wireless Intra-Rumen Unit Calibration Measurements

To check the initial wireless intra-rumen sensor unit calibration, methane and carbon dioxide concentrations measured by the infra-red Dynament® gas sensors are taken wirelessly and validated by gas chromatography in the Queensland Bioscience Precinct (QBP) laboratory. During the experiment different levels of methane, or carbon dioxide, are injected into a sealed glass vessel containing the wireless intra-rumen sensor node shown in Figure 2. Gas concentrations are taken by the wireless intra-rumen sensor and a gas chromatograph (GC).

3.3.3 Gas Diffusion Rates through the Siloxane Membrane

The initial wireless intra-rumen sensor node, shown in figure 2, is fitted into the diffusion cell made from a CRD and a polydimethyl siloxane membrane as shown in Figure 6. The device is installed in a fistulated sheep for 6 hours and then removed and held in fresh air for a further 14 hours. The methane and carbon dioxide concentrations measurements are then download wirelessly from the intra-rumen sensor unit.

3.3.4 Dynament® Gas Sensor Intra-Rumen Drift Measurements

From previous experiments, zero calibration drift of the infra-red sensors was observed and recorded after approximately two to five days in the rumen. Therefore a series of experiments were undertaken to locate the drift problem in the Dynament® gas sensors that were attached to a single wireless intra-rumen sensor unit within a siloxane mounted CRD capsule.

In the first experiment, two new Dynament® carbon dioxide sensors in the CRD capsule were placed in a water bath at a temperature of 39^{o} C and at high humidity levels. The surrounding atmosphere was spiked with carbon dioxide gas at the 63^{rd} minute. The two carbon dioxide concentration measurements were then download wirelessly from the intra-rumen sensor unit. In the second experiment, the two sensors in the CRD were tested in the rumen of a fistulated sheep and left for 41 hours before downloading the carbon dioxide measurements. In the third experiment, two Dynament® carbon dioxide sensors which have been used previously for intra-rumen experiments and have sensor drift, were used in a fistulated sheep to record data 30 hours.

3.3.5 Initial Wireless Intra-Rumen Sensor Unit Radio Range Measurements

The initial wireless intra-rumen sensor unit radio telemetry system was designed to allow transmission of data from grazing animals in the field to a laptop computer via a USB connected Nano sync node. For the experiment a wireless intra-rumen sensor unit was installed in a fistulated steer. The animal was then taken to an open paddock and measurements of radio connectivity, in metres, were taken for different heading angles (z axis) from the rumen opening. The experiment uses a 7dBi gain 916 MHz antenna on the laptop Nano sync node.

3.3.6 Initial Wireless Intra-Rumen Unit Battery Life Calculations

The expected battery life of the initial wireless intra-rumen node shown in Figure 2 was calculated. The batteries used in the calculation were the AA 4.5 volts @ 2.4A hour. The gas sampling duty cycle from two minutes to 24 hours is used for the calculation.

3.3.7 E2v® Methane Sensor Calibration Measurements

To check the e2v® dual sensor calibration, methane concentrations were measured using the glass vessel, sensor cabling, e2v® evaluation circuit board and laptop as shown in Figure 8 and 18. Gas concentration measurements are also taken by gas chromatography (GC) in the Queensland Bioscience Precinct (QBP) laboratory to validate the results. During the experiment different levels of methane were injected in the sealed glass vessel containing the e2v® dual sensor. Gas concentrations were taken by the laptop and the GC. The zero and span values are calculated from the e2v® methane gas readings.

3.3.8 E2v® Siloxane Membrane Methane Gas Response Measurements

Measurements of the methane gas concentration, through the siloxane membrane used on the diffusion cell, were evaluated. The apparatus includes the glass vessel, sensor cabling, e2v® evaluation circuit board, diffusion cell and laptop as shown in Figure 8 and 18. Initially methane was injected into the vessel to a concentration of 20.1% as measured by gas chromatography. Over time the methane gas concentration rose from 0 to 20.1% in the diffusion cell as measured by the e2v® dual gas sensor. From the results a best fit exponential curve was calculated giving an approximate time constant for the siloxane membrane methane gas response.

Figure 18. Equipment used to perform in vitro testing of the e2v® dual gas sensor

3.3.9 E2v® Siloxane Membrane Methane Gas Measurements with Activated Carbon

To study the effects of activated carbon on methane concentrations in the diffusion cell, methane concentrations was measured as described in 3.3.8. Activated carbon (18.0g) was installed in a pouch around the e2v® dual sensor. Methane gas was injected into the glass vessel in the range from 0 to 36.4% and the e2v® methane gas concentration measurement was taken after it is stabilised within the diffusion cell. The glass vessel concentration is also recorded using GC.

3.3.10 New Wireless Intra-Rumen Sensor Unit Intra-Rumen Measurements

In this experiment the e2v® methane and carbon dioxide gas sensor was attached to the new wireless intra-rumen sensor unit. The unit was then fitted, along with 9g of fresh activated carbon, into the siloxane mounted diffusion cell and installed in a fistulated sheep for 3 or 6 days. The methane and carbon dioxide concentration measurements and a number of other measurements were then downloaded from the intra-rumen sensor unit.

3.3.11 New Wireless Intra-Rumen Node Battery Life Calculations

The expected operation life of the device calculated for a range of sample rates, from every 30 seconds (continuous) to once per day was evaluated and the results shown in figure 24. This calculation assumes a battery capacity of 3000mAh which reflects the specifications of the Ultrafire 3.7V, 3000mAh Li-ion (18650) battery used by the new wireless sensing device. Currently the gas sensor is energised for 30 seconds prior to obtaining the sensor reading to ensure the value is stable.

3.3.12 Oxymax Calorimeter System and Respiration Chambers

CSIRO Livestock Industries has 5 sheep respiration chambers in operation associated with an Oxymax Calorimeter at the F D McMaster Laboratory, Armidale. The system is capable of measuring carbon dioxide, methane, oxygen and hydrogen sequentially from a predetermined number of chambers in use for experimentation.

Sheep are placed in the chambers for a period of 24 hours and individual gases are measured every 5-10 minutes depending on the number of chambers being operated and total gas emissions are calculated for the period.

The Oxymax System is calibrated regularly at the start of new experiments with a certified gas mixture standard [20.9% O_2, 1.08% CO_2, 0.11% CH_4 and 0.051% H_2] and adjustments made to the individual gas analysers when calibration or zero drift is evident.

Before animals are placed in the respiration chambers with feed and water, the flow rate of air through the chambers is adjusted to 150-160 litres per minute. The various gas analysers are powered up 60 minutes before the commencement of the experiment and the system sample pump and sample drier pump are switched on 1-2h before the start.

Experimental Design for Validation of Rumen Sensors:

The feed ration for experimental sheep consists of Ridley pellets with a composition of lucerne 500, wheat 100, pollard 200, bran 175, salt 20 and ammonium chloride 5 g/Kg DM. The ration equates to 18.5-20.0% crude protein with an energy content of 8.9MJ/kg DM.

Estimated sheep pellet requirements for animals at various body weights were calculated to be 800g/d for a 40kg animal maintenance indoors and 960g/d for a 50 kg animal under maintenance conditions.

Schedule:

Week 1

1. (-) 48h commence feeding expt. Ration (50% maintenance to 2 fistulated sheep and 100% maintenance to the other 2 fistulated sheep [adjusted to live weight of sheep ranging from 40.0kg to 45.0kg]
2. Take feed sample (100g) for analysis
3. (-)24h place devices in the rumen of sheep set for 0.5h gas readings
4. (-) 1h move sheep from A/H to respiration chamber facility
5. Weigh out rations and set up chambers with both feed and water
6. Set chamber readings for 0.25h for 4 chambers
7. Place sheep in chambers 1-4
8. Run expt. for 23 hours
9. The following morning empty waste trays and repeat rations for the second time period (24-47h)
10. The following morning empty waste trays and repeat rations for the second time period (48-71h)
11. Return sheep back to animal house and feed 100% maintenance rations

Week 2

1. Feed sheep on maintenance rations to re-equilibrate

Week 3

2. Repeat the above schedule with the rations reversed (i.e. animals on 100% maintenance receive 50% and animals on 50% receive 100% maintenance)
3. Recharge batteries in rumen sensors
4. Take feed sample (100g) for analysis

Results and Discussion

3.4 Dynament® Gas Sensor and Initial Wireless Intra-Rumen Unit Experiments

3.4.1 Early Dynament® Gas Sensor Prototype Intra-Rumen Measurements

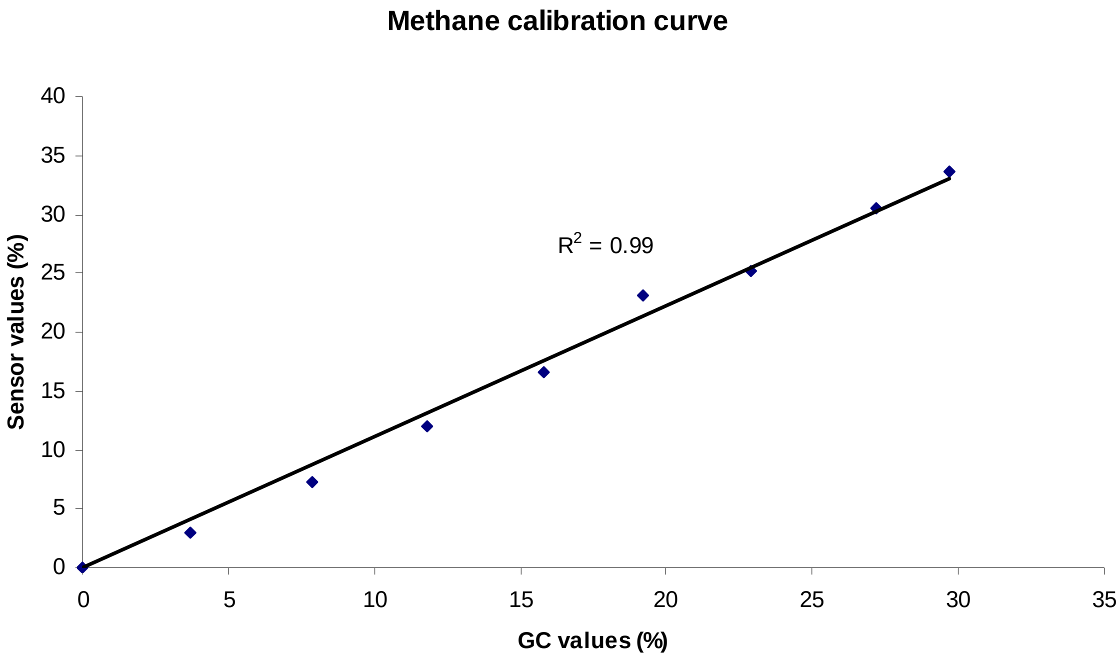
Measurements of gas concentration taken from the rumen of a steer using Dynament® infra-red methane and carbon dioxide gas sensors are shown in table 1. These results from table 1 confirm measurements of methane and carbon dioxide can be made in the rumen, although the time to reach equilibrium was slow. The gas diffusion rates across this material were to be too slow to provide beneficial data on rumen gas levels but demonstrated a proof of concept. The project team then went on to investigate other materials and membranes that were vastly more dynamic and reached equilibrium in a much shorter time period for inclusion in the next generation of prototypes of the technology.

Table 1. Gas diffusion rates into a polyethylene container in the rumen of a grazing steer.

Day	Carbon dioxide (%)	Methane. (%)
1	13.5	4.0
2	19.0	21.0
3	21.0	7.0
4	21.5	7.5
7	25.5	10.0

3.4.2 Initial Wireless Intra-Rumen Sensor Unit Calibration Measurements

Measurements of gas concentration, using the initial wireless intra-rumen sensor node shown in figure 2 were recorded for both methane and carbon dioxide. Measurements of gas concentration were also taken using Gas Chromatography in the Queensland Bioscience Precinct laboratory. The results are shown in Figure 19. The CO_2 readings are at the lower limit of the settings for the gas chromatograph especially when values drop below 10%.


Methane calibration curve

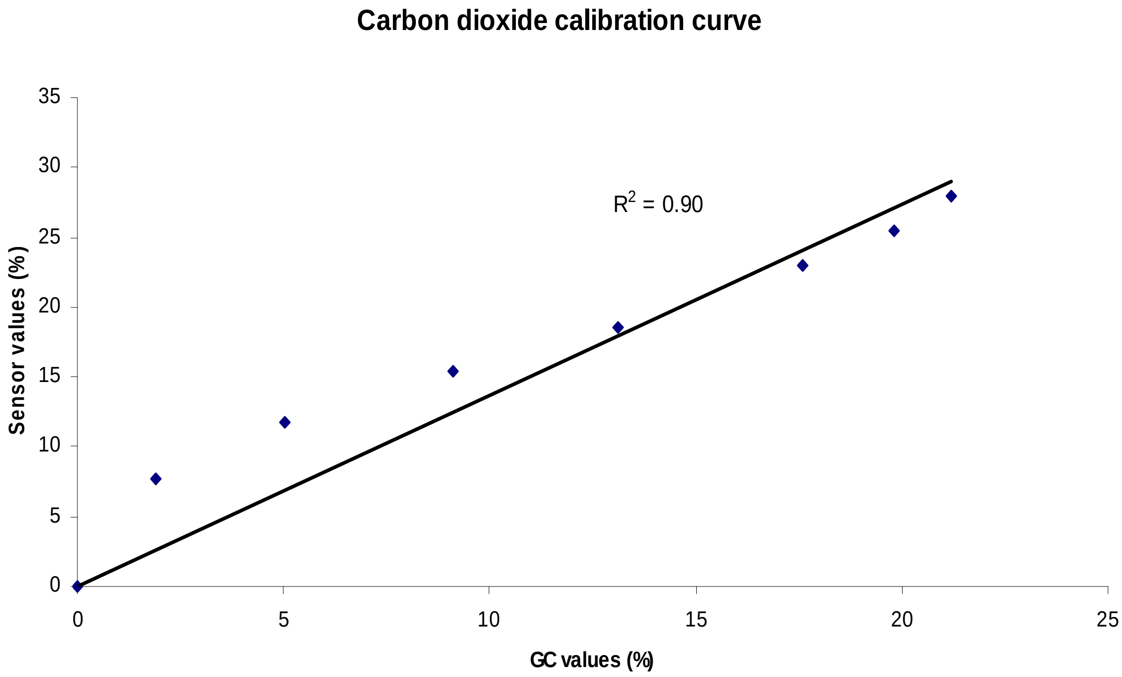

Carbon dioxide calibration curve

Figure 19. Methane and carbon dioxide concentrations measured by Dynament® electronic infrared sensors and validated by gas chromatography in the laboratory at the Queensland Bioscience Precinct, Qld.

3.4.3 Gas Diffusion Rates through the Siloxane Membrane

Measurements of methane and carbon dioxide gas concentrations, using the initial wireless intra-rumen sensor node fitted into the diffusion cell, are shown in figure 20. The device was placed in a fistulated sheep for the first 6 hours and then retrieved and held in fresh air for the remaining 14 hours. The graph shows that the carbon dioxide gas concentration reached equilibrium and returnss to zero much faster than methane gas concentration. Gas diffusion across the CRD siloxane is therefore much faster for carbon dioxide gas than the methane gas.

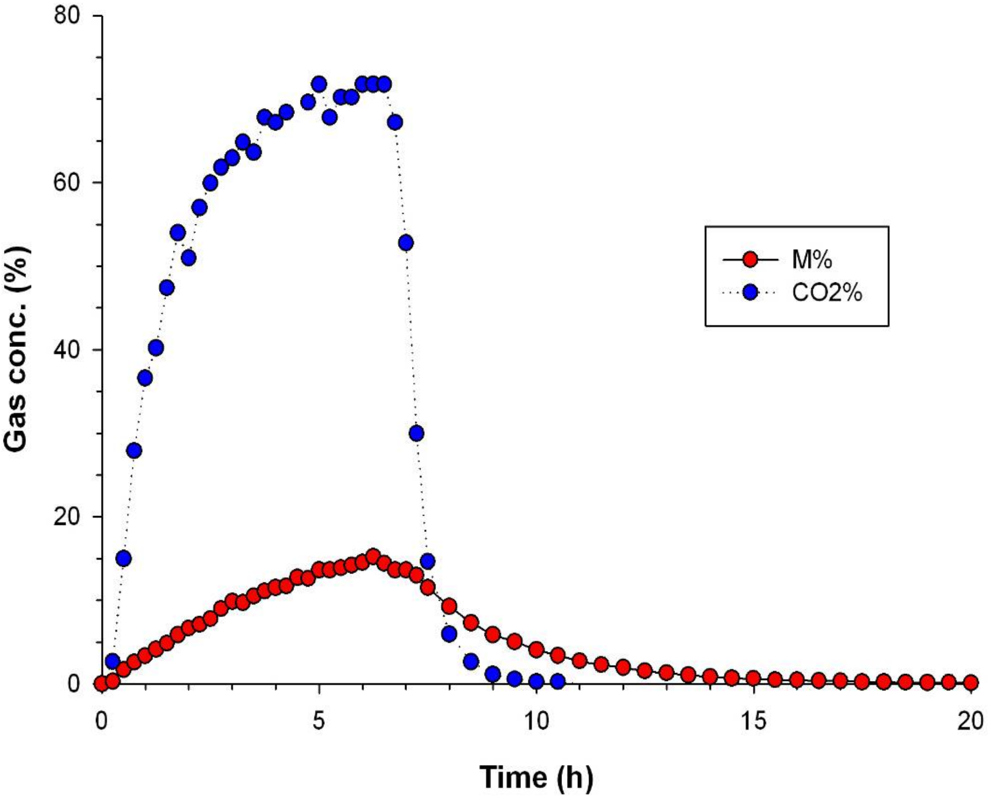


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Figure 20. Gas diffusion rates (CO_2, blue; CH_4, red) through a siloxane membrane mounted onto a CRD cattle device placed into the rumen of a fistulated sheep and removed from the animal after 6.5h.

3.4.4 Dynament® Gas Sensor Intra-Rumen Drift Measurements

There were a number of experiments conducted to investigate the Dynament® Intra-rumen gas sensor drift problem. In the first experiment, two new Dynament® carbon dioxide sensors are attached to a single wireless intra-rumen sensor node within a siloxane mounted CRD capsule in a water bath at a temperature of 39 C and at high humidity levels. The results are shown in figure 21. There was a rise in the recorded carbon dioxide concentration levels when the surrounding atmosphere is spiked with carbon dioxide at the 63rd minute and there is a resultant peak and slow decline after 650 minute. This indicates the new Dynament® carbon dioxide sensors are functioning as per the manufacture's specifications.

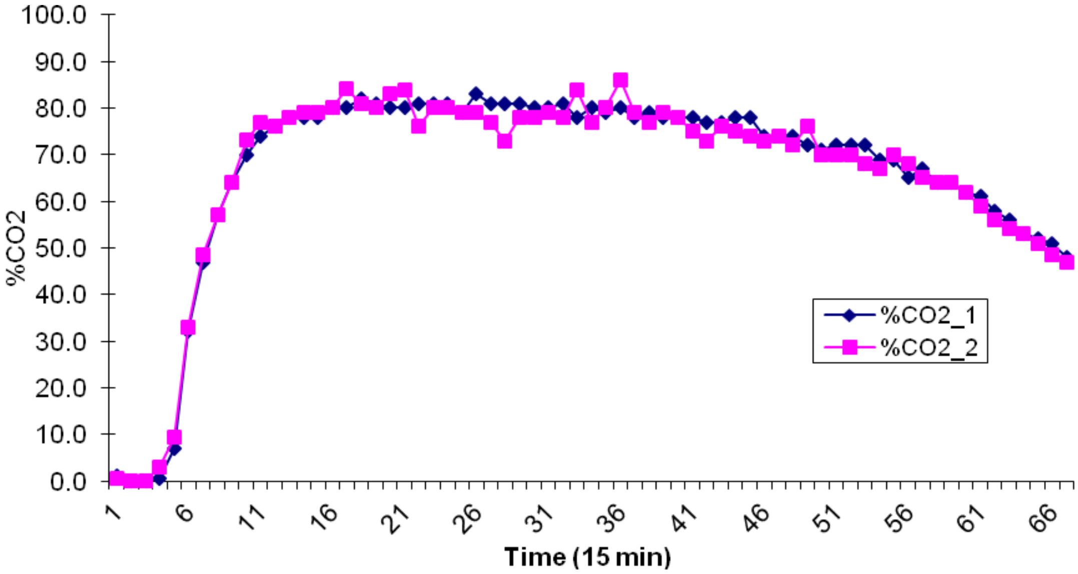

Figure 21. Two new Dynament® carbon dioxide sensors attached to a single node within a siloxane mounted CRD capsule in a water bath at 39°C and at high humidity levels.

In the second experiment, two new Dynament® carbon dioxide sensors in the same CRD capsule were placed in a fistulated sheep. There was a very close correlation between the two gas sensors up to 34 hours (65 samples) where the drift appears in the concentration readings (Figure 22). This indicated the new Dynament® carbon dioxide sensors were not functioning correctly at this point and needed recalibrating.

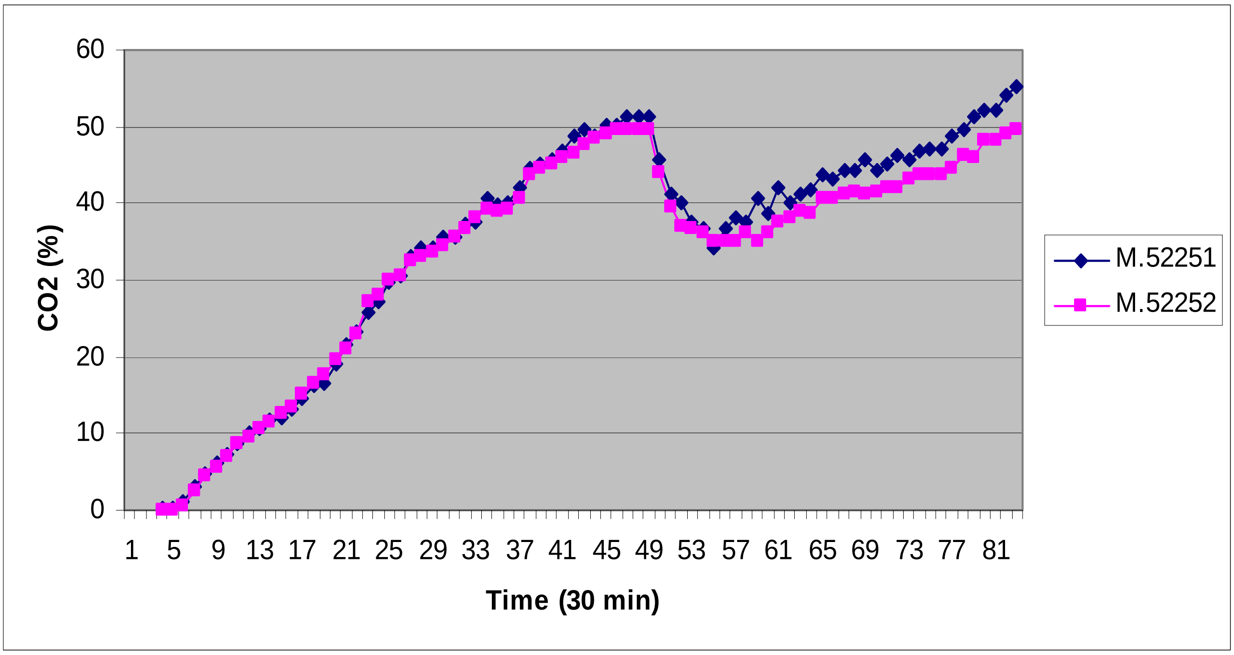

Figure 22. Two new Dynament® carbon dioxide sensors attached to a single node within a siloxane mounted CRD capsule in a fistulated sheep.

In the third experiment Dynament® carbon dioxide sensors, used previously for intra-rumen experiments were placed in a fistulated sheep. The results are shown in figure 23. Both Dynament® carbon dioxide sensors performed poorly and gave inconsistent readings after 3 hours (6 samples) including readings of gas concentration above 100%. This indicated the Dynament® carbon dioxide sensors were faulty. Dismantling the gas sensor, as shown in figure 7, showed the sensor internal surfaces weree contaminated by water vapour and hydrogen sulphide.

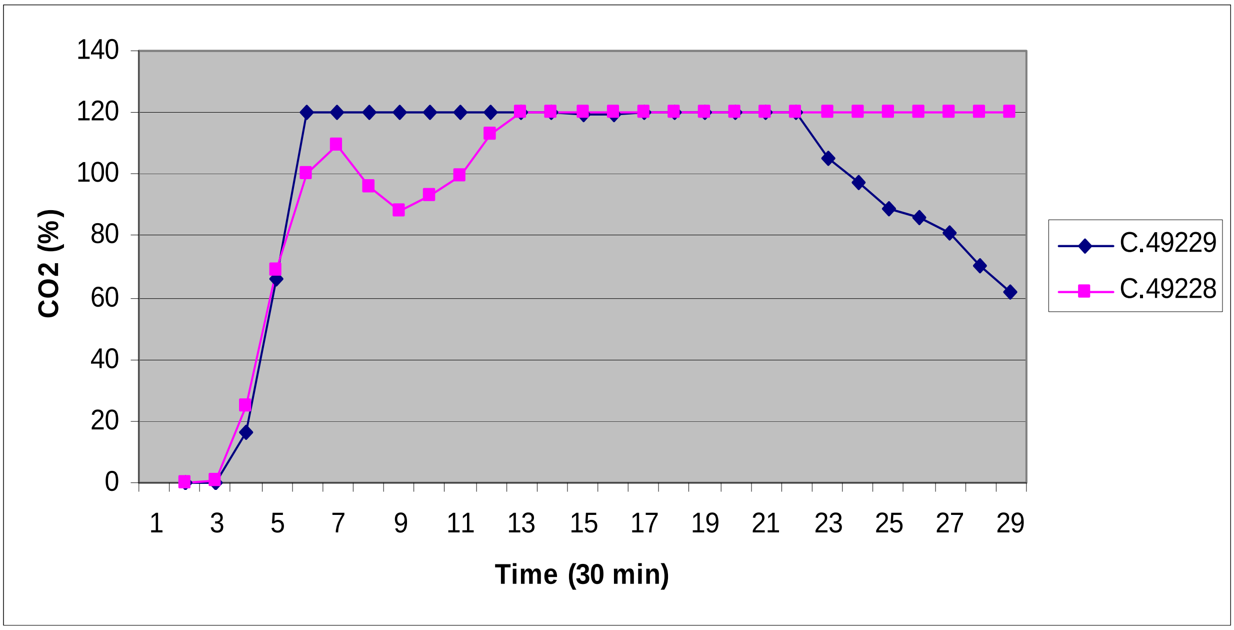

Figure 23. Two Dynament® carbon dioxide sensors, used previously for intra-rumen experiments, attached to a single node within a siloxane mounted CRD capsule in a fistulated sheep.

3.4.5 Initial Wireless Intra-Rumen Node Radio Range Measurements

The initial wireless intra-rumen sensor node was installed in a fistulated steer and from an open paddock the measurements of radio connectivity are shown in table 2, which are taken for different heading angles (z axis) from the rumen opening. The radio signals from the device within the rumen were picked up over a range of 30 to100m. The distance of transmission depended on the position of the animal relative to the antenna receiver. Greatest distance achieved was observed when the animal was at 90 degrees to the base antenna and with the rumen facing this point. The gas sensor device has both a radio transmitter and a receiver. This allows the operator to make changes to the sampling mode remotely whilst the animal is still at pasture without the need to yard the experimental animal.

It was determined that a four static node system in the paddock with 50 m spacing would provide good connectivity to the wireless intra-rumen sensor device (Table 2).

Table 2. Measurements of radio connectivity from a wireless intra-rumen sensor node, installed in the rumen of a fistulated steer, to a Nano sync node with a 7dBi antenna.

Clockwise angle to rumen opening (degrees)	Distance to animal with a 7dbi antenna (metres)
0	105
45	35
180	30
315	45

3.4.6 Initial Wireless Intra-Rumen Unit Battery Life Calculations

The expected life of the initial wireless intra-rumen node battery is shown in figure 24. The batteries used in the calculation are the AA 4.5 volts @ 2.4A hour. Given a gas sampling rate from 2 minutes to 24 hours the resulting battery life is one day and less than one week respectively. The calculations indicate a completely new platform and new gas sensors are required to improve the battery life expectancy of the wireless intra-rumen nodes.

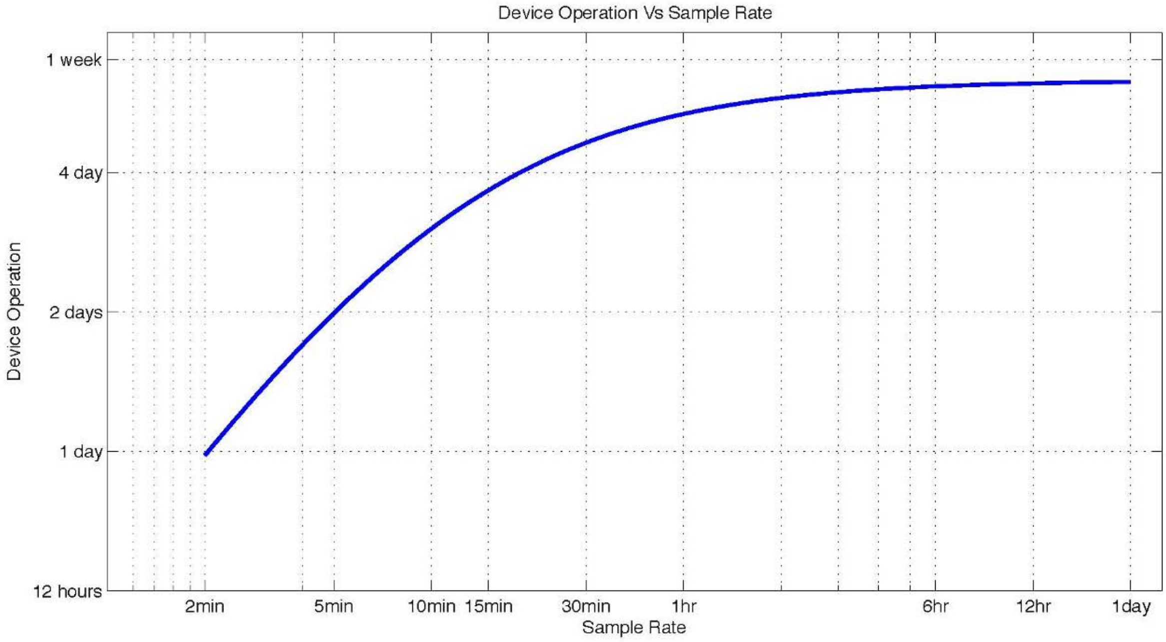

Figure 24. Battery life expectancy of the initial wireless intra-rumen node versus the gas sample rate.

3.5 E2v® Gas Sensor and Final Wireless Intra-Rumen Node Experiments

3.5.1 E2v® Methane Sensor Calibration Measurements

The measurements of methane gas concentration using the glass vessel, sensor cabling, e2v® evaluation circuit board and laptop are shown in figures 8 and 18. Measurements of methane gas concentration are also taken using GC at the Queensland Bioscience Precinct laboratory. The methane gas concentration measured by GC showed very good correlation with e2v® dual gas sensor estimates (Table 3).

Table 3. E2v gas sensor methane concentration versus gas chromatography methane concentration measurements

e2v Methane concentration (%)	GC Methane concentration (%)
0	0
8.03	7.78
20.29	19.86
24.35	24.72
43.54	43.54

3.5.2 E2v® Siloxane Membrane Methane Gas Response Measurements

The measurements of methane gas concentration, through the siloxane membrane used on the diffusion cell are shown in figure 26. Initially methane was injected into the vessel to a concentration of 20.1% and after 6 hours the methane gas concentration level in the diffusion cell reached 98% of the final value. The best fit exponential curve, also shown on figure 26, has a time constant of 90 minutes (5400 seconds) and is a close match to the diffusion cell response.

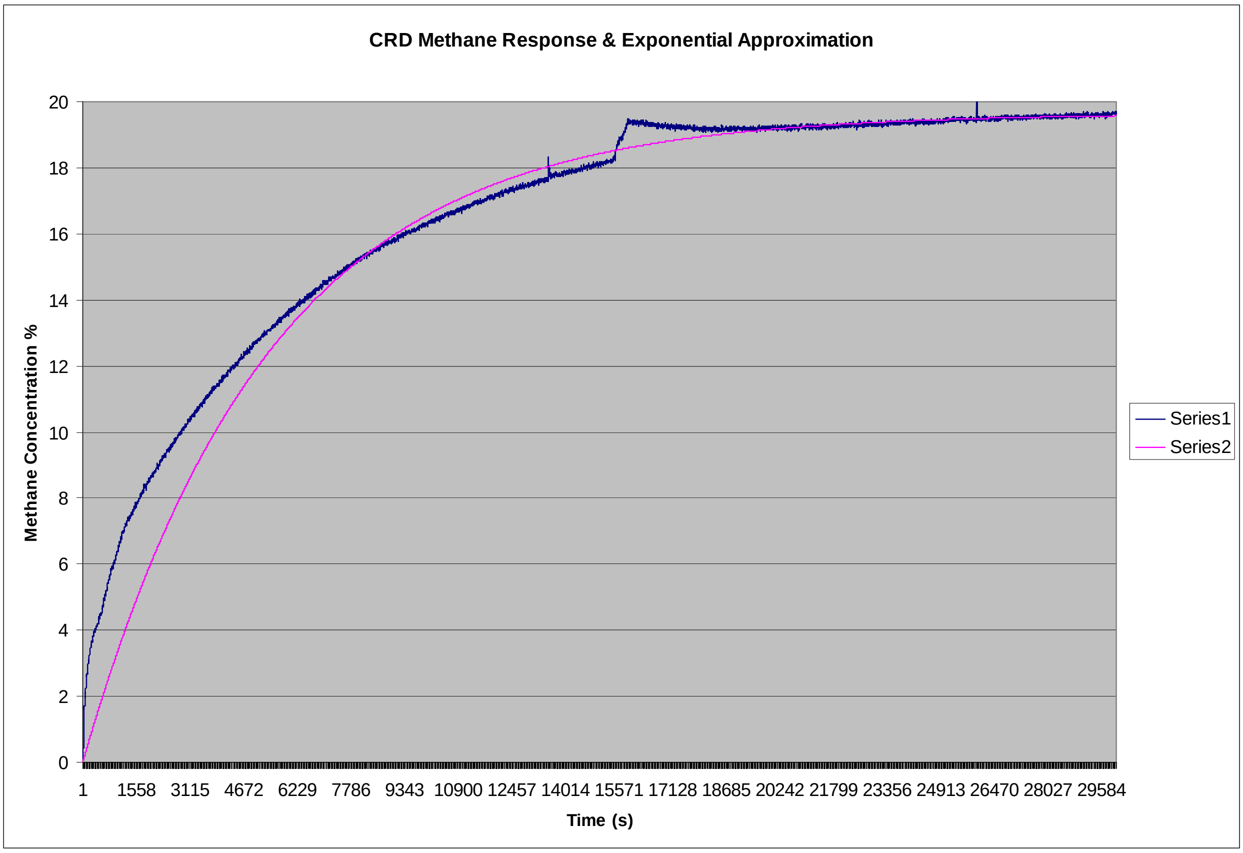

Figure 26. Gas diffusion across the CRD siloxane membrane diffusion cell (blue line) verses the best fit exponential curve (pink line).

The algorithm for the exponential fitted curve is:

$C=(C_{out} - C_{int})(1-e^{(-t/T)})+C_{int}$ (1)

Where;

t is the time in seconds

T is the time constant in seconds

C is the measured methane concentration (%)

C_{out} is the outside methane concentration (%)

C_{int} is the initial methane concentration (%) at t = 0

3.5.3 E2v® Siloxane Membrane Methane Gas Measurements with Activated Carbon

Activated carbon has been shown to significantly reduce the deleterious effects of water vapour and hydrogen sulphide on the infra-red gas sensors by acting as a sink and binding the gas in the short to medium term. Following successful tests of the e2v® dual gas sensors in fistulated sheep in the presence of activated carbon there was a need to study the impact of activated carbon on methane concentrations.

Measurements of methane concentrations in the diffusion cell containing 18g of activated carbon are shown in Table 4. Methane gas was injected into the glass vessel in the range from 0 to 36.4% and the e2v® methane gas concentration measurements were taken after they stabilised within the diffusion cell. The results indicate a correction factor can be applied to the e2v® methane gas measurements when activated carbon is included in the diffusion cell.

Table 4: E2v® dual gas sensor in the diffusion cell with activated carbon and the external GC methane concentration measurements

E2v® Methane Concentration in the CRD with Activated Carbon (%)	External GC Methane Concentration (%)	Correction Factor
0	0	1.0
6.18	6.42	1.039
17.77	19.78	1.113
29.89	36.44	1.219

3.5.4 New Wireless Intra-Rumen Sensor Node Intra-Rumen Measurements

The new intra-rumen wireless sensor unit was used in a number of intra- rumen experiments in fistulated sheep (Figure 13). In all of the experiments 9g of fresh activated carbon is placed within the diffusion cell.

In the first experiment, the new intra-rumen wireless sensor unit was installed in the fistulated sheep for 3 days. The e2v® methane and carbon dioxide gas sensor concentration and rumen temperature measurements are shown in figure 27. The initial gas concentration level rise for carbon dioxide is much steeper than that of methane; this confirms the faster response of carbon dioxide gas compared to methane gas though the siloxane diffusion cell. The increase in rumen temperature each day is a consequence of fermentation following feeding. There is a corresponding increase in carbon dioxide gas concentration with a relative decrease in methane gas concentration.

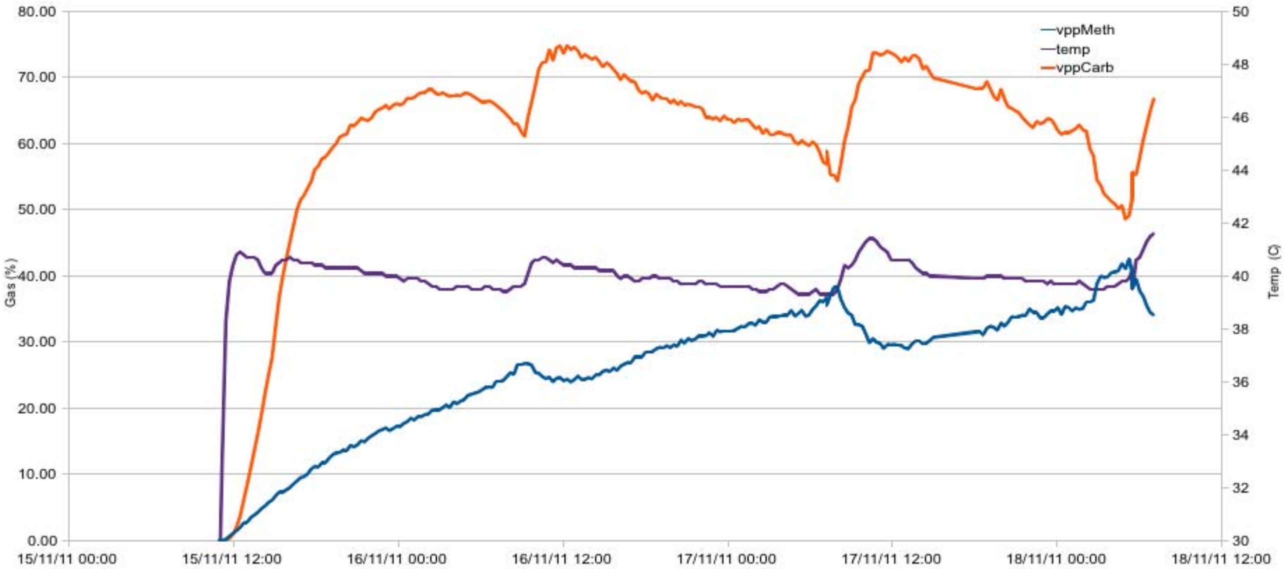

Figure 27. New wireless intra-rumen sensor node with E2v® dual gas sensor within the siloxane diffusion cell in a fistulated sheep for 3 days (Top line, CO_2; Bottom line CH_4; Middle line , temperature).

In the second experiment, the new intra-rumen wireless sensor node was installed in the fistulated sheep for 6 days. The e2v® methane and carbon dioxide gas sensor concentration and rumen temperature measurements are shown in Figure 28. The same pattern of increase in rumen temperature, as a consequence of fermentation following feeding, and the corresponding increase in carbon dioxide gas concentration and decrease in methane gas concentration is evident in the graph.

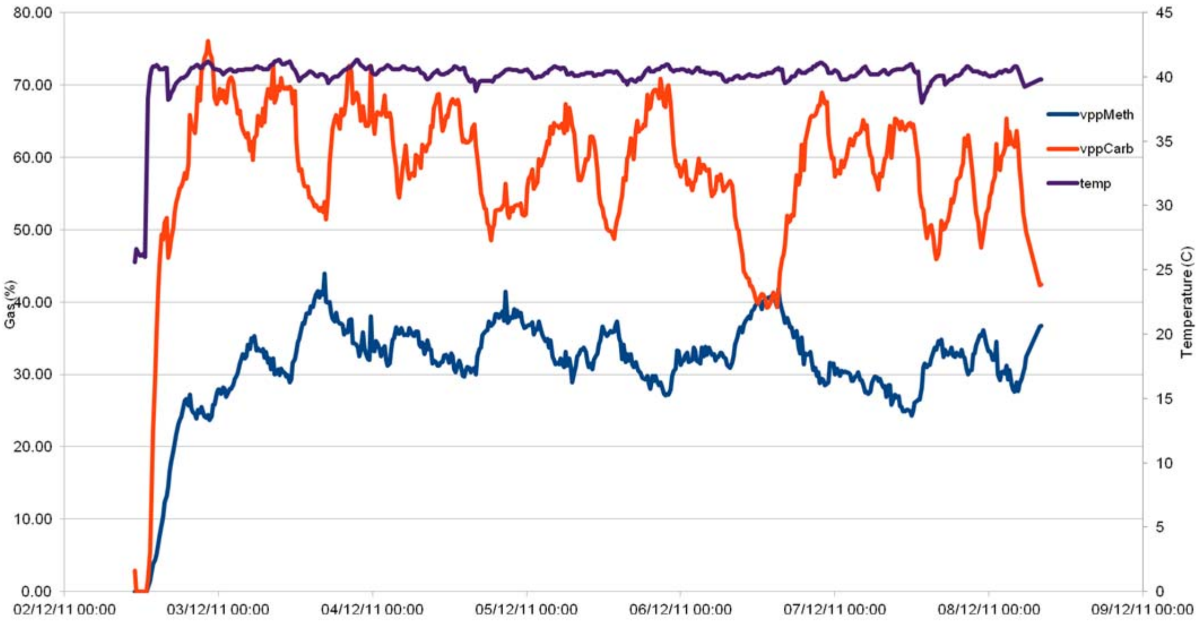

Figure 28. New wireless intra-rumen sensor node with E2v® dual gas sensor within the siloxane diffusion cell in a fistulated sheep for 6 days (Top line, temperature; Middle line , $CO_{2;}$ Bottom line CH_4).

3.5.5 New Wireless Intra-Rumen Node Battery Life Calculations

The expected operation life of the device was calculated for a range of sample rates from every 30 seconds (continuous) to once per day, and is shown in figure 29. This calculation assumes a battery capacity of 3000mAh which reflects the specifications of the Ultrafire 3.7V, 3000mAh Li-ion (18650) battery used by the new wireless sensing device. Currently the gas sensor is energised for 30 seconds prior to obtaining the sensor reading to ensure the value is stable.

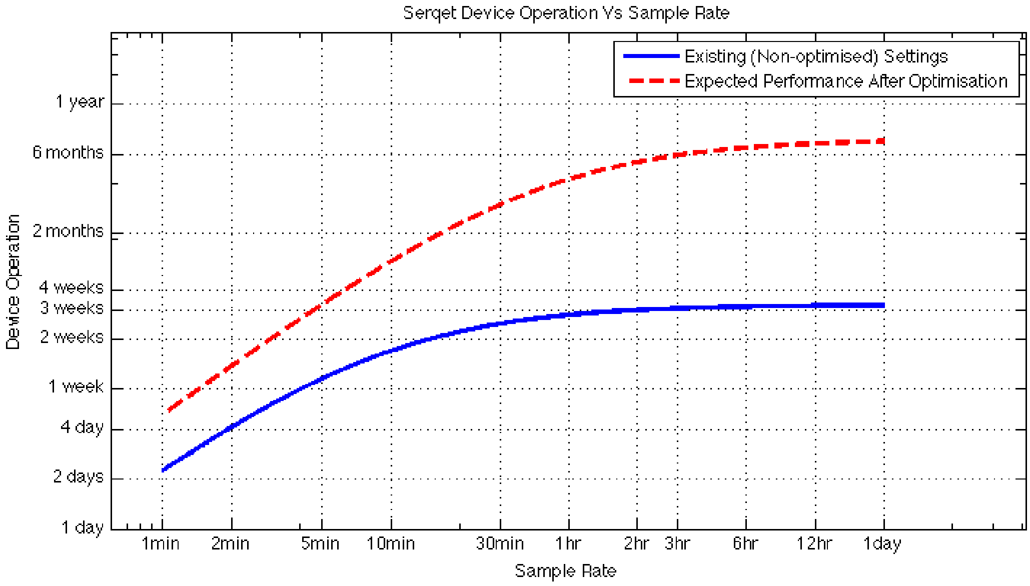

Figure 29. Predicted battery curves based on existing settings (5mA CPU + 60 sec settling time) and the anticipated future setting (0.5mA CPU + 30 sec settling time).

3.5.6 Oxymax Calorimeter System and Respiration Chambers

To date, it has not been possible to achieve a data set of respiration chamber emissions in parallel with the intra-ruminal device measurements due to delays in dealing with calibration and zero drift with the gas sensors. These issues were unforseen and led to significant delays in reaching a prototype device that is accurate and repeatable over time. The result of the improvements is the development of the most recent prototype with greater capabilities and functionality than first envisaged.

4 Success in Achieving Objectives

4.1 Latest prototype

4.1.1 Progress on a fully functional device

The device is presently capable of detecting diurnal variations in carbon dioxide and methane concentrations in the rumen of experimental sheep in relation to feeding patterns. In addition, rises in temperature within the rumen due to fermentation can be monitored by the latest device. Although measurement of absolute gas concentrations utilizing algorithms to predict emissions from gas concentrations is still some way off, at this point in time, steady progress is being made in meeting this objective in project B.CCH.1021. Additional time is required to develop relationships between gas concentrations with actual emissions. A shift in gas levels has been detected with the intra-ruminal device in the same animal fed above and below maintenance rations.

A joint provisional patent has been lodged in the last 6 months covering the invention and intellectual property associated with the development of the device titled “System, method and device for measuring a gas in the stomach of a mammal”.

Significant advances have been achieved at this point in time in the development of a modified CRD diffusion cell to house the electronic components, the software underpinning the system including wireless telemetry as well as advanced capability and functionality of this novel technology.

5 Impact on Meat and Livestock Industry – now & in five years time

5.1 Impact on Meat and Livestock Industry

The livestock industry has invested large amounts of time and funds into developing mitigation strategies for reducing ruminant greenhouse gas emissions, particularly methane emissions. However, in order to develop, monitor and validate such mitigation strategies it is necessary to be able to readily measure enteric gas emissions from large numbers of individual animals. It is desirable to measure gas emissions in an autonomous fashion which does not significantly disturb or impede the animals in their natural grazing environment.

The most widely adopted technique for such free-ranging methane measurements in individual animals involves estimating the rate at which livestock exhale methane using a sulphur-hexafluoride (SF6) tracer gas. However, such tracer based measurement techniques have been found to generate relatively inaccurate readings. For example, some tests have shown large variability in recordings between and within animals when measured on consecutive days. The current development of wireless rumen sensor (bolus) technology for use in grazing ruminants provides exciting opportunities for the future. This technology is relatively easy to use and provides an excellent and affordable option for continually measuring rumen gases.

The immediate impact of this project on the meat and livestock industries will be the ongoing opportunity to have an impact on reducing methane emissions from livestock with potential for further development through round 2 of the MLA/DAFF Reducing Emissions from Livestock Research Program that will commence in 2012.

The Australian livestock sector which produces the greatest amount of enteric methane is the beef cattle industry. There are approximately 22 million beef cattle in Australia and about half of this population is found in northern Australia. Northern Australian production systems are typified by low rates of gain (< 1 kg/day), high turn-off ages (~ 3.5 years old), lack of grain for finishing, and an almost complete reliance on low quality forage based diets. It has been estimated that as little as a 5% increase in the efficiency of digestion could yield an economic benefit of at least $100 million to the cattle industry. The excretion of methane from the rumen can represent a loss of 8-10% of the energy of the diet depending on the type of diet. A reduction in methanogenesis in the rumen can be associated with improvements in feed conversion efficiency without affecting intake. Therefore reducing methane production could benefit the ruminant energetically provided the efficiency of ruminal metabolism is not compromised.

Research from the current project is likely to have impact on both the intensive and extensive sectors of the ruminant livestock industries particularly if there are productivity benefits associated with a reduction in methane emissions. If successful, the technology will allow researchers to discriminate phenotypic differences and the effects of variation in feed base, climate and management on methane emissions. This should lead to feeding management strategies for improved utilisation of the feedbase and a reduction in methane emissions from grazing and intensively fed ruminants. The technology also offers the opportunity to identify genetic variation between animals in intensity of methane production under different grazing systems. Although quality and amount of feed are strong factors in driving methane production, there is also variability between animals in the degree of methane that they produce per unit of feed, with examples of clear outliers of animals that produce less methane than other animals in the population at different levels of feed intake. However, what is not known is whether animals that are more productive produce less methane, nor is it known by what mechanism low methane producers achieve this end. The intra-ruminal methane sensor will be a powerful tool to advise whether genetic selection for low methane production and superior liveweight gains is feasible using genetic measures of the animal that could be employed commercially. The technology has the potential to provide a major benefit in screening cattle for high and low methane emissions under normal grazing conditions without the need to handle or modify their behavioural patterns during the testing period. It would be considered by industry and the general public as a very low stress system on animals as well as being far less intrusive than the current options available for estimating methane emissions from grazing ruminants.

6 Conclusions and Recommendations

Although the technology has not reached maturity, major advances have been made during the course of the project despite major technical problems that have arisen as the device has progressed from laboratory to in-vivo evaluation in ruminants. Miniaturized sensors and sensor networks are already playing a significant role in manufacturing industries and are used for environmental monitoring of landscapes in conjunction with satellite imaging. It is now becoming more evident that these sensor technologies have a role to play in agricultural systems, either on or within the animal. As more research groups focus on developing and enhancing sensor systems, then the combined knowledge gained during this process has the potential to accelerate sensor technology outputs.

The provisional patent application on this technology provides MLA and CSIRO protection to further develop the technology in the short to medium term without the threat of other research groups from competing in the same space and utilizing the intellectual property generated from this joint research project.

Project B.CCH.2021 which is an extension of this project to deal with the problems and issues outlined earlier in this report provides the opportunity to continue this research to the next phase whereby the opportunity exists to determine the relationship between methane and carbon dioxide concentrations in the rumen with absolute and quantifiable emissions of these gases from ruminants.

Building on the substantial achievements and developments to date of this new and novel technology requires further research work to be done in order to achieve the final objective.

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8 Appendices

8.1 Appendix 1

TECHNICAL SPECIFICATIONS FOR FLECK™ 3B & NANO

FLECK™ 3B

The FLECK™ 3B is the latest in our sensor node family.
Its specifications are:

- 50 x 60 mm
- processor:
    - Atmel Atmega 1281
    - 128 Kb program flash
    - 8 Kb RAM
    - TinyOS and FOS operating system
    - 3 LED indicators
    - onboard temperature sensor
    - onboard realtime clock (allows for deep sleep mode)
    - onboard 1 Mb flash memory, upgradeable to 4 Mb.
- radio:
    - Nordic RF 905
    - 433/915 MHz with GFSK modulation
    - 50 kilobits per second
    - SMA Antenna, range >1000 metres.
- power supply:
    - 3.5 – 8 V
    - 33 µA standby current
    - onboard solar cell battery charger with monitoring of solar voltage, solar charge, current and battery terminal voltage
    - supports rechargeable batteries (3 x AA) with overcharge protection.
- screw terminals:
    - 4 digital I/O, interrupt, counters, PWM generator
    - 2 analog inputs.
- expansion board interface:
    - 2 x 20 way connectors
    - robust 4 x hole mounting.
- signals:
    - most Atmega 128 digital I/Os
    - 8 x ADC pins
    - hardware SPI bus.
- 3.3 V power can be switched on/off by the processor datamate connector:
    - 2 x RS232.

NANO

The Nano is 25 x 20 millimetres and has an indoor range of 7 – 20 metres.
It consists of:

    - onboard 8051 microcontroller

- EEPROM
- three axis accelerometer
- Nordic 915 MHz transceiver.
- It runs on a minimal version of FOS.