Ruminant methane – Extension of the animal calorimetry facility at AgResearch Grasslands

PROJECT CODE: CC MAF POL_2008-28 (139-4)

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Summary:

Work to provide New Zealand with world class facilities for the rapid and highly accurate measurement of methane (CH_4) emissions from forage-fed ruminants has been completed. Four new calorimeters for sheep and a prototype chamber for cattle have been built and tested. The four new sheep chambers and the existing four chambers have been incorporated into a state-of-art eight-chamber sheep calorimetry and data acquisition system. This sheep system is now fully operational and is capable of measuring not only CH_4 emissions but also CO_2 and H_2 emissions as well as O_2 uptake. Innovative software which enables variable times of sample switching allows rapid cycling between samples (< 5 min, for 8 sheep and one ambient sample) producing a robust number of data points for the calculation of gas emissions. Historically, H_2 emissions from ruminants have been neglected. Hydrogen is the principal energy source for rumen methanogenesis, but occasionally H_2 can be emitted in respired breath. The system will further our understanding of CH_4 production by measurement of H_2. The cattle calorimetry system has been designed and built to high standards. Despite its large volume and weight, it provides an air-tight enclosure with safety provisions for both the animal and the operator. Both the sheep and cattle calorimetry systems include animal safety measures in compliance with animal welfare regulations. There is a plan in place for an additional cattle calorimeter to achieve a 2-chamber system. This work provides the facility and resources to advance and accelerate research to reduce CH_4 emissions from forage-fed ruminants by enabling research in a timely and replicated fashion without excessive delays in access to facilities. No other facility of such nature exists in the Southern hemisphere.

PROJECT CODE	**CC MAF POL_2008-28 (139-4)**
PROJECT TITLE	**RUMINANT METHANE - EXTENSION OF THE ANIMAL CALORIMETRY FACILITY AT AGRESEARCH GRASSLANDS**
RESEARCH LEADER	**CESAR PINARES**

Framework Report

Business/Institution: AgResearch Limited

Goal:

To construct four sheep calorimeters as well as a prototype calorimeter for cattle to provide New Zealand with world class facilities for the rapid and highly accurate measurement of methane (CH_4) emissions from forage fed ruminants.

Context of the project:

The extensive developments in inventory and mitigation research on enteric CH_4 emissions carried out in New Zealand during the last 10 years have been based on the use of the sulphur hexafluoride (SF_6) tracer technique. Compared to calorimetric techniques, the tracer technique is associated with larger between-animal and within-animal variability in CH_4 emission estimates, which are a cause of concern when accurate assessments of emissions from individuals are required. Whole-animal enclosed calorimetric chambers are the 'gold standard' when accurate measurements of total digestive tract CH_4 emissions (via eructation and flatus) are required. In August 2007 AgResearch commissioned a 4-chamber calorimetry facility for sheep, which has been fully utilised since that date. However, the 4-chamber facility was restricted to simultaneous measurements on a small sample size of sheep, leaving limited scope for the testing of new CH_4 mitigation products or new approaches to CH_4 research with appropriate replications. In addition, despite the fact that cattle are the most important source of CH_4 emissions, no facilities have been available in New Zealand for accurate measurements of emissions from this source.

Approach:

Objective 1: Construction of 4 sheep calorimetry chambers

The four new sheep calorimetry chambers were a copy of the existing chambers, constructed mainly by AgResearch engineers and are in the Reid-Ulyatt PC2 facility. The new chambers function according to the same principles as the existing chambers which run at a slight negative internal pressure, so there are no air leaks outwards of the chamber. The chambers are comfortable, safe, and allow the animals to lie down and stand up at will. The transparent construction material facilitates visual contact between animals housed in the different chambers.

The four existing chambers and the four new ones have been integrated into one single 8-chamber calorimetry system (**Fig. 1**). This allows continuous and simultaneous measurements of methane emission from 8 animals and the background ambient air. The new system has an extended capability for measurements of other respiratory gases (carbon dioxide, oxygen and hydrogen).

Fig 1. The sheep calorimetry facility at AgResearch Grasslands. The newly built 4 chambers (to the right) and the previously existing 4-chamber facility (to the left) are managed as sub-units (for purposes of air circulation only), and integrated into an 8-chamber facility. Fresh air is sourced from a common vent in the building ceiling.

The volume of each chamber is 1.8 m^3 (1.8 m long, 0.85 m wide and 1.2 m high). The frame is made of 25 × 25 × 3 mm square section aluminium tube with a 3 mm thick aluminium sheet floor. The walls and roof are 6 mm clear polycarbonate sheet, fixed to the aluminium frame using silicone sealant and rubber seated screws to form an air tight seal. The chambers have both front and rear access doors fitted with internal rubber seals. The front door is locked by two solenoid locks which open automatically in the event of either a power failure or failure of the air circulation system. The rear door is locked by three adjustable latches. They are mounted on castors with four levelling feet. The sheep are restrained in the chambers in modified metabolic crates with a feed bin, drinking water container and separate trays to collect faeces and urine (**Fig. 2**).

Fig.2. Sheep are housed in modified metabolic crates with faecal and urine collection trays. The transparent walls of chambers enable the sheep to acclimatise to the system almost immediately, as shown by their eating, ruminating and lying behaviour.

Each chamber is fitted with a fresh air inlet at the front and an air outlet at the back and air flow is ducted via flexible (38 mm O.D.) polyurethane hoses. The air inlets are piped from a common ceiling vent in the building (Fig. 1). The outlet hose from each chamber is connected to a 38 mm air filter (SVCC198, P.V.R. srl, Valmadrera, Lecco, Italy) and then to a diaphragm gas meter (AL425, American Meter Company) for wet gas flow measurement (**Fig. 3**). As the four ‘existing’ and four ‘new’ chambers are deployed in two rows within the room (a PC-2 facility), each row is managed as a separate sub-unit for purposes of air circulation. Thus, within a row, all the four outlets from chambers and individual gas meters are connected to a common pump (**Fig. 4**). Two air pumps (UNI-JET 40, ESAM, Parma, Italy) assembled in parallel provide air circulation throughout the chambers at continuous but adjustable flow rates (100 – 250 L/min). Air (enriched with respiratory gases) from the pump outlets is exhausted outside the building.

Each chamber is fitted with two internal ventilation fans for efficient mixing of expired gases and incoming air (**Fig. 3**). Sensors (Vaisala Oyj, Helsinki, Finland) for measuring relative humidity and temperature (Vaisala Humicap® HMT100) and barometric pressure (PTB 110) are fitted in each chamber (**Fig. 5**) so the air flow data can be adjusted to dry, standard temperature and pressure conditions.

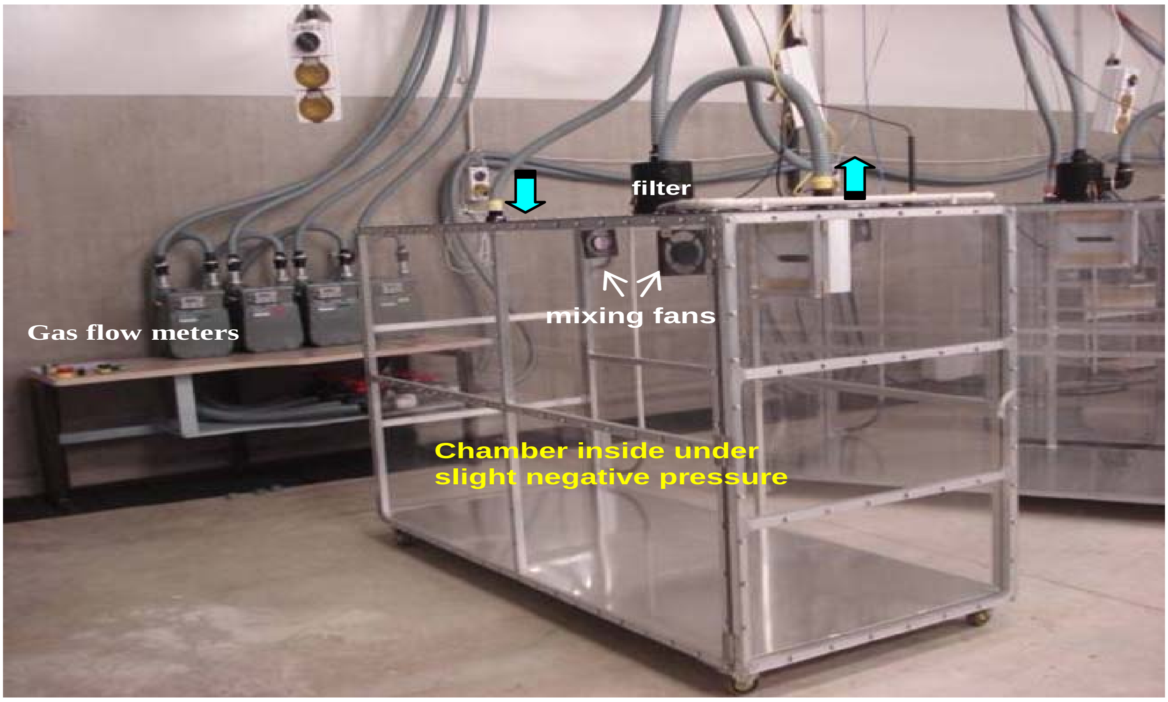

Fig. 3. Sheep calorimetry chamber showing its air inlet and outlet (in light blue arrows), internal mixing fans, air filter and piping to and from gas flow meters.

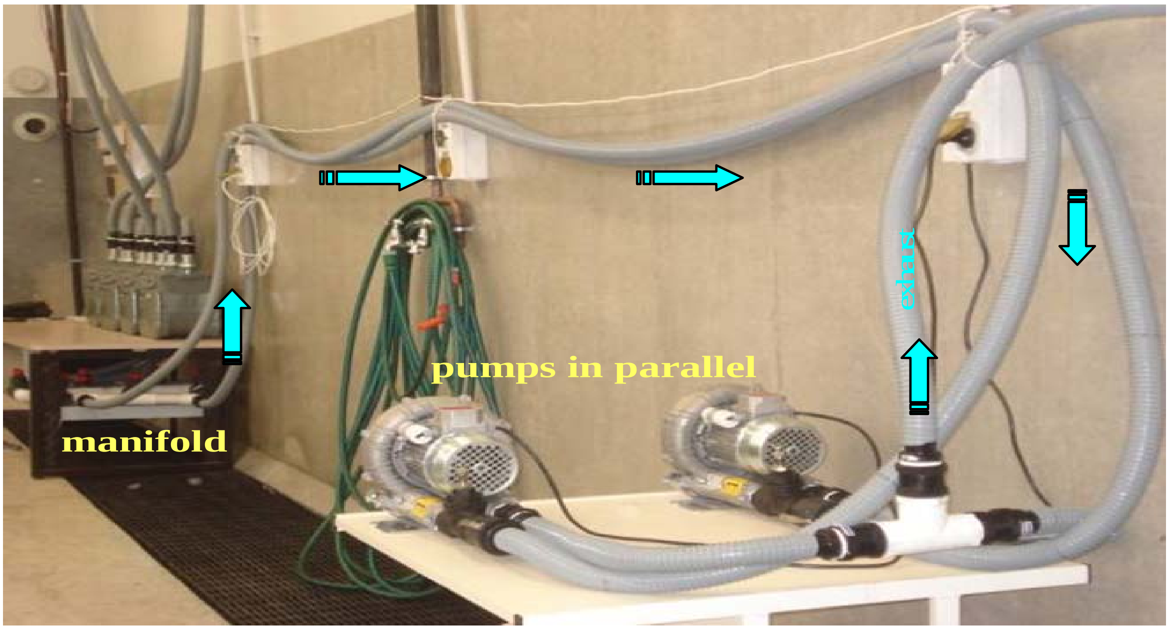

Fig. 4. Gas pumping system: A gas manifold at the outlets of four flow meters is connected to parallel-assembled vacuum pumps. Outlets from the vacuum pumps have a common exhaust pipe to outside the building. Arrows indicate direction of air flow.

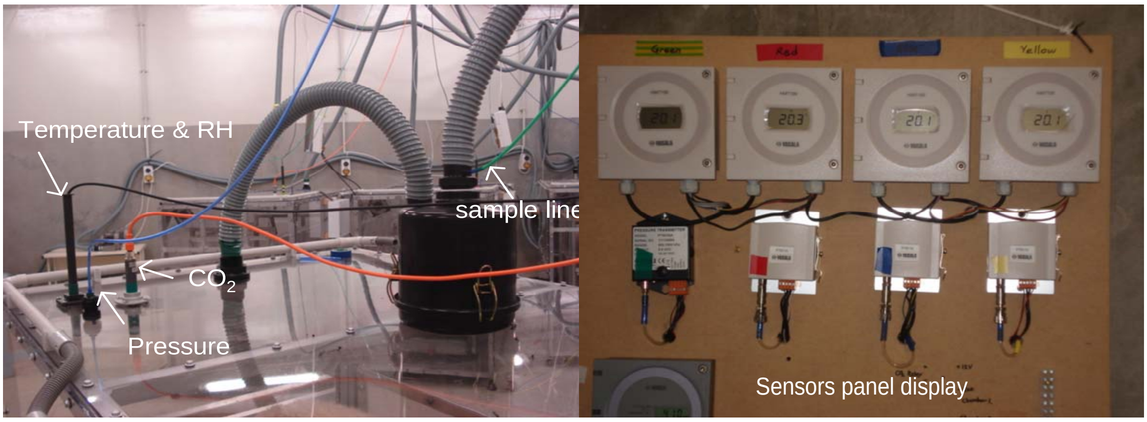

Fig. 5. Chamber environment sensors (temperature, relative humidity and CO_2 concentration) and display of instantaneous conditions. One CO_2 sensor is utilised per set of 4 chambers.

Outlet gas from each chamber (exiting the gas filter, **Fig. 5**) is continuously sampled at 2 L/min into a multiport 10 channel gas switching unit (S.W. & W.S. Burrage, Ashford, Kent, UK) (**Fig. 6**) using 1/8 inch polyurethane tubing with an in-line 7 µm filter. Innovative software developed at AgResearch allows the multiport unit to switch the samples taken from each of the 8 chambers and an ambient sample at variable times based upon the stability of the concentration readings (standard deviation of the last three readings < 1 ppm). The time taken to separately measure methane concentration from all the chambers plus the ambient is typically less than 5 min. Before entering the analyser the sample is filtered through a 0.5 µm pore filter and dried using heated (MDH-110-96, Perma Pure, New Jersey) and un-heated (MD-110-24P-2, Perma Pure, New Jersey) gas dryers. A gas drying unit (DDS-001-240, Nexus Analytical Pty. Ltd., Engadine, NSW, Australia) supplies dry air at 4.0 L/min as counter flow to the sample drying units (**Fig. 6**). Sample gas is delivered to the gas analyser by a micro diaphragm pump (N86KTE, KNF Neuberger Inc, Freiburg, Germany) at 2 L/min flow.

A multigas analyser (4900C Continuous Emission Analyser, Servomex Group Ltd., East Sussex, UK) (**Fig. 6**) with capability for measurement of methane (CH_4), carbon dioxide (CO_2) and Oxygen (O_2) is used for the gas analyses. A hydrogen (H_2) sensor (7HYT Citicels, City Technology Ltd., Hampshire, UK) has also been incorporated into the multigas analyser compartment. The principle of CH_4 and CO_2 detection is the non-dispersion infrared technology, whereas for O_2 the paramagnetic principles are used and an electrochemical cell determines H_2 concentration. The multigas analyser is set for measurements of gas concentrations in the following range: CH_4, 0–200 ppm; CO_2, 0–2000 ppm; O_2, 0–21%; H_2, 0–50 ppm. The accuracies of measurements for CH_4, CO_2, O_2 and H_2 are 0.5 ppm, 50 ppm, 0.05% and 5 ppm, respectively. The multigas analyser is calibrated every morning using 99.99% pure nitrogen as a zero (baseline) and a standard gas mix containing 200 ± 4 ppm CH_4, 2000 ± 20 ppm CO_2, 21.1 ± 0.1 % O_2 and 50 ± 1 ppm H_2 with N_2 balance(BOC Limited, New Zealand).

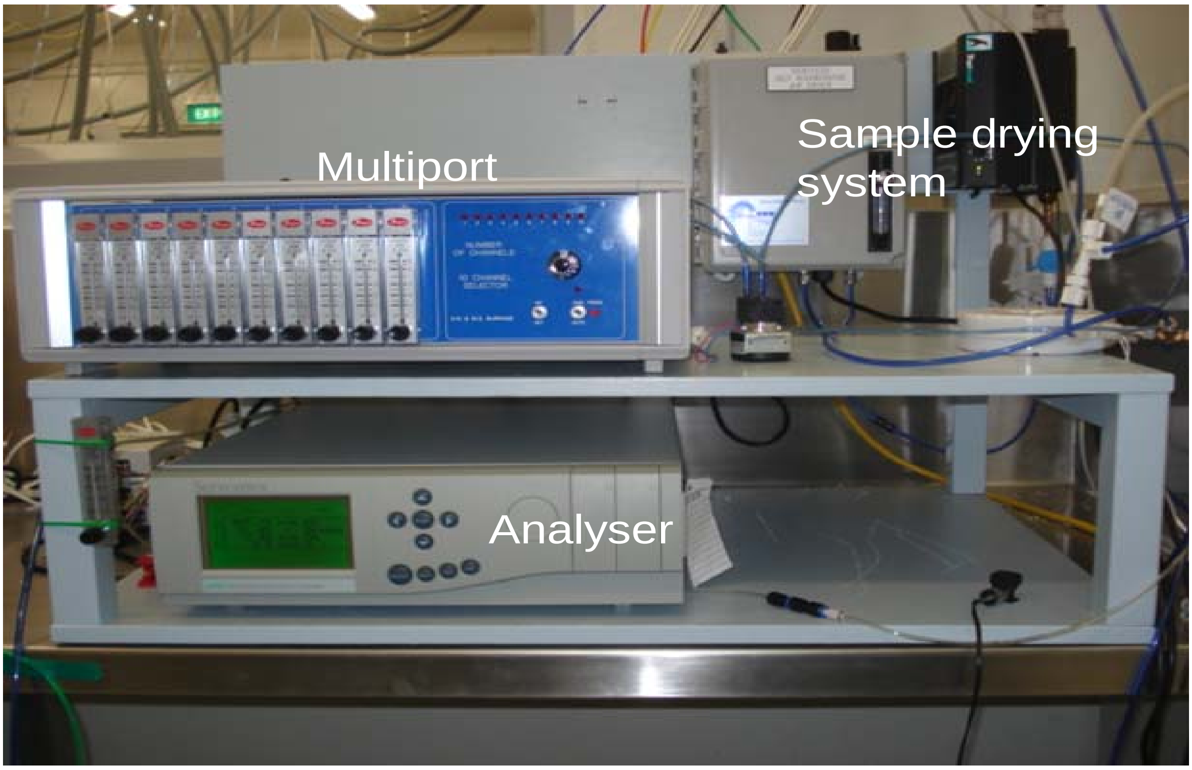

Fig. 6. A gas multiport delivers sample gas from each chamber at variable intervals. Sample is filtered and dried before entering the multigas analyser.

The data acquisition system (see display, **Fig. 7**) records data for gas concentrations and gas conditions every 5 seconds providing between 4 and 12 readings per sample. However, only the last of the readings is considered to be most accurate and used for emissions calculations. Instantaneous gas concentrations (above the ambient) in conjunction with the specific standardised gas flows are used to calculate instantaneous gas emissions (or uptake in the case of O_2) at 2.7– and 5.0 min. intervals. Total daily emissions are calculated as area under the curve of data points.

The calorimetry chambers operate under a slight negative pressure so that no respiratory gases are lost from the system. Consequently, there is a risk that power failure or malfunctioning of air pumps could cause suffocation of the animals if the air flow ceased. Safety measures to remove this risk include; the front door of the chamber (with powered solenoids) will open automatically in the case of a power failure (**Fig. 8**), the parallel assembly of the two vacuum pumps (**Fig. 4**) maintains air circulation even if one pump fails. In addition, air circulation outlets from the four chambers are piped into a single vacuum system, and one chamber is fitted with a highly accurate CO_2 sensor (GMP222, Vaisala Oyj, Helsinki, Finland) (**Fig. 5**). This configuration means that any failure in the air flow will increase CO_2 concentrations in all chambers, and when this reaches 4000 ppm power will be cut to the solenoids, releasing the front doors of the four chambers. The calorimetry system has alarms which indicate abnormal gas concentrations (high CO_2 and CH_4, and low O_2) and environmental conditions (temperature, relative humidity and pressure) which are connected to the AgResearch computer network to facilitate remote monitoring of the system. In the event of an alarm or emergency the operator's response is within 10 min.

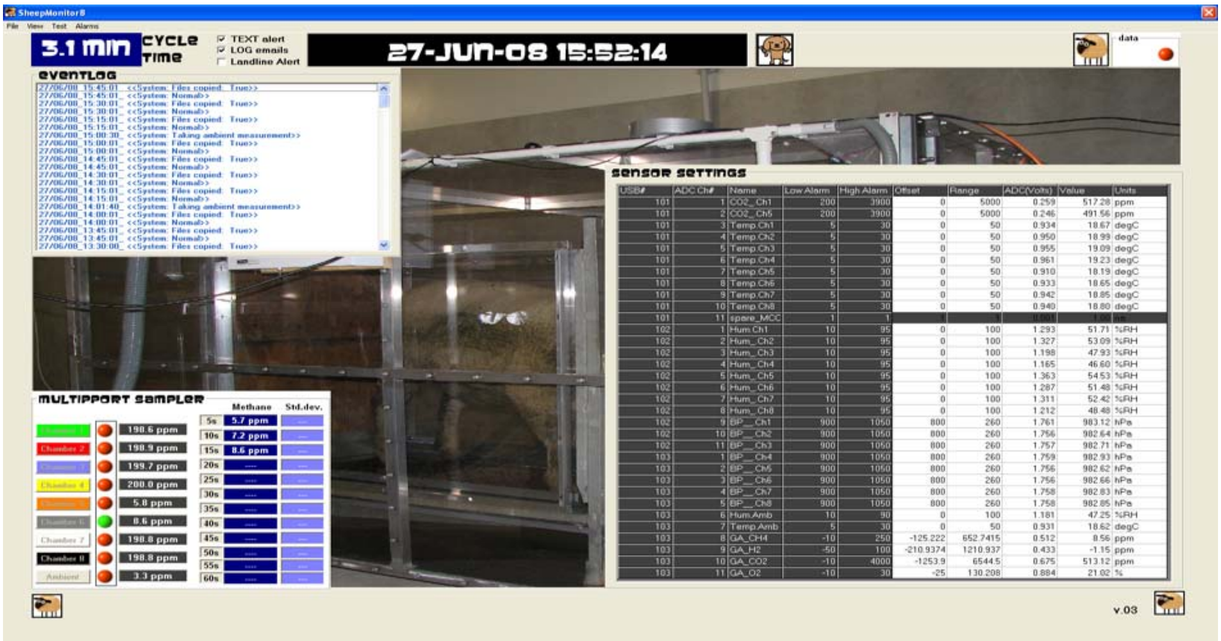

Fig. 7. On screen display of the data acquisition system, alarms monitoring and cycling time (< 5 min) of measurement of concentrations on all samples (8 chambers + 1 ambient).

Fig.8. Front doors of chambers fitted with solenoids open in case of power failure or high CO_2 (4000 ppm)

The respiratory chamber system has been tested by metering ultra pure CH_4 using a metered flow of 20 mL/min, to deliver the mean concentration of about 100 ppm. These tests revealed recovery rates of CH_4 between 97 and 100%. Tests that calculate the recovery of the other gases measured in the calorimetry system are currently in progress.

Objective 2: Construction of prototype calorimeter for cattle

A state-of-art prototype cattle calorimeter chamber has been designed and built by the AgResearch engineering staff and it operates under the same principles as the sheep calorimetry system. The chamber is constructed from mild steel rectangular hollow sections (RHS) and like the sheep calorimeters is covered in clear 6 mm thick UV stable polycarbonate sheet making this a very sturdy and safe chamber for both the operator and animal. The net volume of the cattle chamber is 15.8 m^3. It is 4.0 m long, 2.0 m wide and 2.2 m high. Due to its size and building access, it was necessary to construct the chamber in two halves (**Fig. 9**), which allowed it to be assembled within the Reid-Ulyatt PC-2 Facility at AgResearch Grasslands. The two halves were bolted together with a full bead of silicone sealant ensuring a stable airtight seal. This type of construction allows removal of the chamber from the building if required.

Fig. 9. The cattle chamber was built in two equal halves

Fig. 10. Cattle calorimetry chamber showing its structural metal frame and clear walls and roof

The main frame of the chamber uses 70 × 50 × 5 mm steel tube for its superstructure and 50 × 50 × 5 mm steel for the sub-frames, with animal/operator safety dividing partitions fabricated from 30 × 30 × 3 mm steel tube. The floor of the chamber is 6 mm thick mild steel plate welded to the main frame and silicone-sealed. The clear polycarbonate covering walls, roof and doors of the chamber is fixed to the metal frame using neutral-cure silicone sealant and rubber-seated TEK screws at 100 mm centres to ensure air-tightness. The chamber has one front and two rear access doors fitted with rubber seals. The front door (115 cm wide × 215 cm high) which is located at the left side is closed by two electro-magnetic locks. In the event of either a power failure or a CO_2 build-up to predetermined levels, the door locks de-energise, and the doors spring open allowing a fresh air supply to reach the animals. This door is also used for animal feeding.

The two rear doors are hinged on either side of the chamber (**Fig. 10**). The main rear door (115 cm wide × 215 cm high) located at the right side is used for animal access, whereas the smaller door (90 cm wide × 215 cm high) located at the left side is used for operator access.

These doors are secured with three large clamping wing nuts ensuring that an air-tight seal is achieved by the door against the main chamber frame.

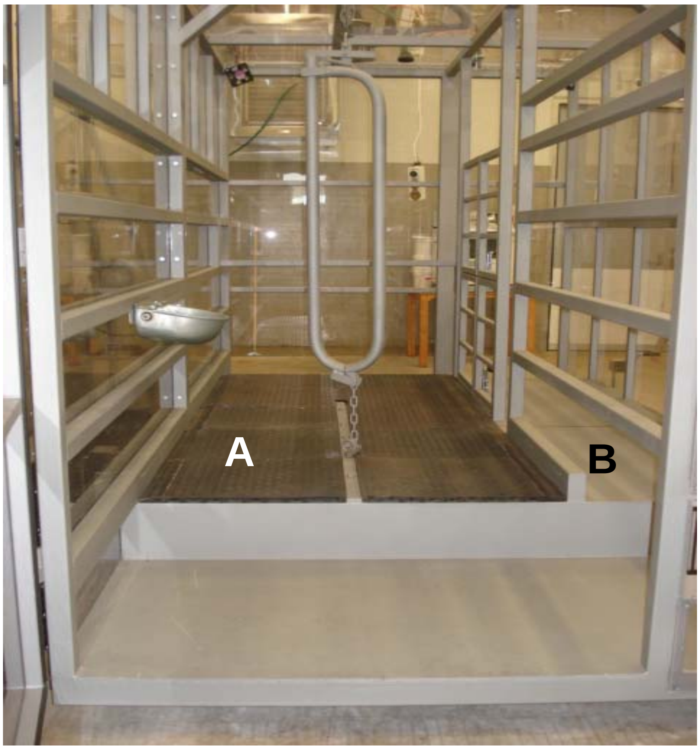

Fig 11. Front view of the cattle chamber showing the low floor for feeding bin placement, the cattle housing area (covered in rubber mat; 'A'), the operator working area ('B') and the internal metal frame dividing cattle and operator areas.

The chamber main floor is an elevated (20 cm) platform, the whole width of the chamber and is 80 cm from the front door and 65 cm from the back doors. The platform comprises two sections (A and B) (**Fig. 11**). Section A (117 cm wide and 250 cm long) is the cattle housing area and is covered in rubber mats. Section B (90 cm wide and 250 cm long) is the operator safety area and is separated from section A by an internal metal frame with 'easy-access' openings for rumen sampling. The front 'low floor' is used for placement of the feeding bin, whereas the back 'low floor' enables placement of excreta collection bins. Drinking water is provided by a water trough located at the right internal wall of the chamber. For reasons of safety and requirements for measuring water intake, the water supply is piped down from a 50 L closed-bucket located on top of the chamber.

A head bail is built into the chamber to prevent cattle from turning around. It allows easy movement of the animal during feeding, drinking, resting and lying down. The rear of the cattle housing area is provisioned with a metal bar at 70 cm above the floor to avoid the animal stepping on the excreta collection bin. The chamber has provisions for adjustment of feeding bin, water trough and head bail to suit different animal sizes (e.g. calves and adult animals). The whole chamber is levelled using four adjusting screws (**Fig. 10**) that can be retracted so the chamber is able to be manoeuvred on large braked castors.

The chamber is fitted with a fresh air inlet in the front section of the ceiling and an air outlet in the rear ceiling. All the piping for air circulation is through flexible polyurethane hose (51 mm O.D.). The air inlet is piped from an air intake in the ceiling of the building. Incoming fresh air and the respiratory gases are mixed by four fans located at the top corners of the chamber. The outlet hose is connected to a 51 mm air filter (F300, P.V.R. srl, Valmadrera, Lecco, Italy) and then to a gas quantometer (Qa100-80-016, Elster/Amco, Malnz-Kastel, Germany) for wet gas flow measurement (**Fig. 12**). The outlet from the gas meter is connected to a manifold and then to a side-channel vacuum pump (SV 7.190/1-01, Gebr. Becker Gmbh & Co., Wuppertal, Germany) using polyurethane hose (**Fig. 12**). The vacuum pump draws air through the chamber at continuous but adjustable flow rates (1000 – 2000 L/min), and when a second chamber is built the air circulation will be provided by two vacuum pumps assembled in parallel. Air (enriched with respiratory gases) exiting the air pump is exhausted outside the building. Relative humidity, temperature and barometric pressure sensors (Vaisala Oyj, Helsinki, Finland) are installed in the chamber as with the sheep chambers for standardisation of the gas flow.

Fig 12. Cattle calorimetry system: gas quantometer and manifold, and the side-channel vacuum pump. Arrows indicate direction of gas flow.

Outlet gas from the chamber (exiting the gas filter) is continuously sampled at a rate of 1.0 L/min into a multiport gas switching unit (S.W. & W.S. Burrage, Ashford, Kent, UK) (**Fig. 13**) using 1/8 inch polyurethane tubing with an in-line 7 µm filter. The multiport unit switches the samples taken from the chamber and the ambient every minute. Sample gas is delivered to the gas analyser by a means of a micro diaphragm pump (NMP 09L, KNF Neuberger Inc, Freiburg, Germany) at 1.0 L/min flow. The sample is filtered through a 0.5 µm pore filter and conditioned using a heated gas drier (MDH-110-96, Perma Pure, New Jersey) which uses dry air as counter flow at 2.0 L/min. A non-dispersion infrared gas analyser (ZKJ-1, Fuji Electric Systems Co., Ltd., Tokyo, Japan) (**Fig. 13**) able to detect CH_4 in the range 0 to 200 and 0 to 5,000 ppm is used for CH_4 analysis. The CH_4 analyser is calibrated using 99.99% pure nitrogen as a zero CH_4 baseline and a CH_4 standard with 170 ± 3 ppm of CH_4 (BOC Limited, New Zealand). The gas analyser has 1 ppm resolution for concentrations in the range 0 to 200 ppm. The data acquisition system records data for CH_4 concentrations and gas conditions every 10 seconds providing six estimates per sample, but considering the time delay between sampling point and stable analysis reading, only the last of the six readings for each sample are used for CH_4 emission calculations. Daily emissions are calculated by averaging the 2-min data points. A Servomex multigas analyser capable to measure CH_4, CO_2, O_2 and H_2, and a gas drying system similar to that for the sheep calorimetry system is being acquired.

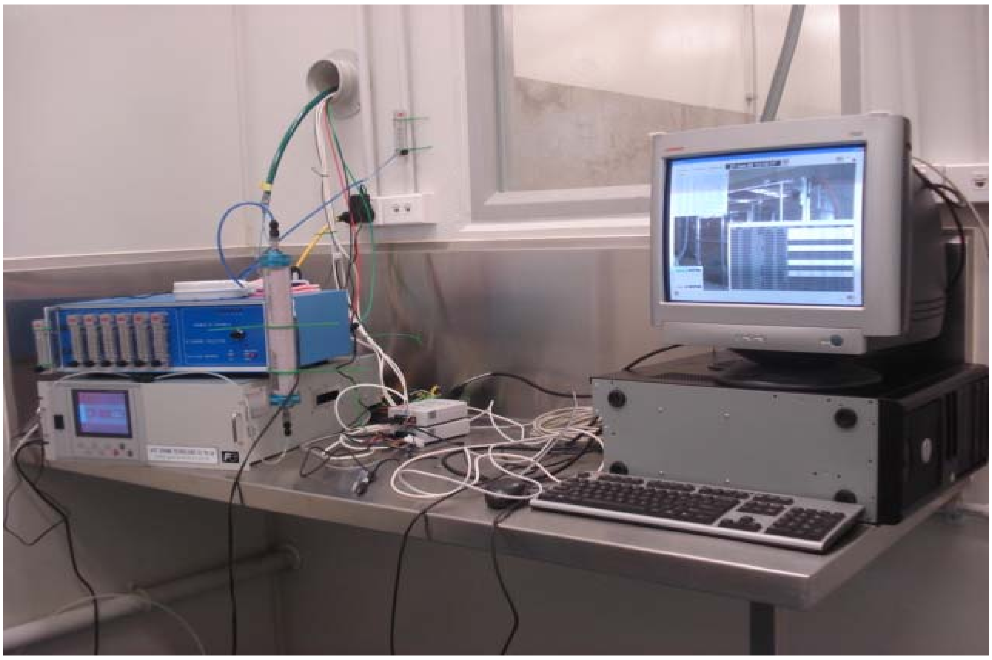

Fig. 13. The cattle calorimetry system operates as a 1-chamber system, but provisions are in place to operate as a 2-chamber system

As in the case of the sheep chambers, the cattle chamber operates under a slight negative pressure so no respiratory gases are lost from the system. Consequently, safety measures to avoid animal suffocation in case of power or system failure are in place (e.g. parallel assembly of pumps, front door fitted with powered solenoids, CO_2 sensor and a comprehensive set of alarms).

The chamber system has been tested by metering ultra pure CH_4 using a metered flow of 180 mL/min, to deliver the mean concentration of about 100 ppm. This test revealed recovery rate of CH_4 in the system of 102%.

The cattle calorimetry system has been tested with animals (dairy cows) (**Fig. 14**). These tests have revealed its robustness and safety for both the animal and the operator. Testing animals accessed the chamber without hesitation and once head-bailed they started eating almost immediately and remained calm. At the end of the testing periods (1, 3 and 6 h) the animals exited the chamber backwards without problems. As result of the animal testing minor modifications in the prototype chamber are required. Modifications include the height of the feeding bin, the protection of the water trough from animal damage and a likely extension of the chamber floor. The cattle calorimetry system has been provisioned to incorporate another chamber. The additional chamber will be commissioned by the end of September 2008. A two-chamber cattle calorimetry system is planned to be operational by October 2008.

Fig. 14. The cattle calorimetry system proved to be safe and friendly for the animal

Outcomes:

The construction of the four new sheep calorimetry chambers has been completed on schedule. These new chambers together with the existing four chambers have been incorporated into a state-of-art eight-chamber sheep calorimetry system. This system is now fully operational and is capable of measuring not only CH_4 emissions but also CO_2 and H_2 emissions as well as O_2 uptake. Innovative software which enables variable times of sample switching allows rapid cycling between samples (< 5 min, for 8 sheep and 1 ambient sample) producing a robust number of data points for the calculation of gas emissions.

The construction, commissioning and testing (gas recovery and animal-friendliness) of the prototype chamber for cattle has also been completed on time. Instrumentation and data acquisition of the cattle calorimetry system was enabled by using existing resources from the original four-chamber sheep calorimetry facility. Current objectives aim to have a two-chamber cattle calorimetry system operational by October 2008 with a state-of-the-art instrumentation and data acquisition system.

Publications:

None (at this time).

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