BEST PRACTICE GUIDELINES
for
HEADBOX USE
to measure
GREENHOUSE GAS EMISSIONS FROM LIVESTOCK

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1. Head Box Construction

1.1 Manufacture of headbox:

The following specifications are based on a headbox using negative pressure. Like all open circuit calorimetry systems, it is possible to either draw the air through or push the air through where the animal is. Drawing air through by suction (a negative pressure system) reduces the risk of animal emissions being lost from the measurement system and reduces the risk of errors due to the headbox not being airtight. In a negative pressure system, the headbox cannot be airtight as air must enter, usually around the neck of the animal, but also through any small gaps or holes elsewhere.

1.2Design, dimensions & materials:

1.2.1 Headbox:

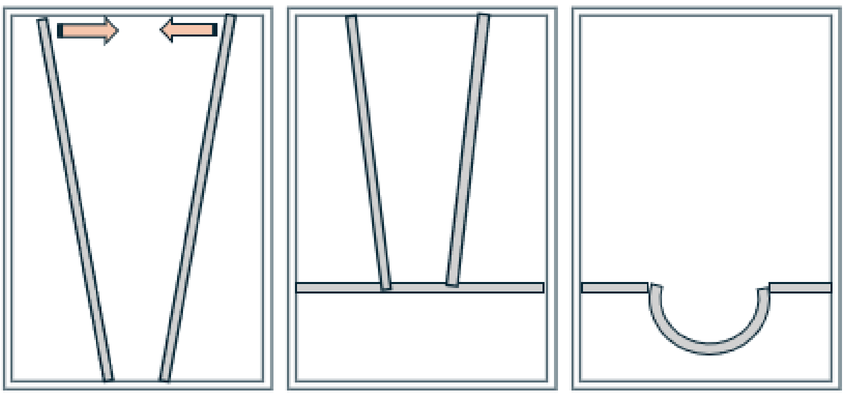
- For large ruminants, the headbox should be approximately 80-100cm Wide, 150-175cm High, 75-90cm Deep, and may be built straight onto the ground or raised from the ground by 25-30 cm. For smaller cattle (such as yellow or Bali cattle, a smaller headbox is appropriate).
- For small ruminants, the headbox should be approximately 50-60 cm Wide, 75-100cm High, 40-60 cm Deep.
- The headbox should consist of a steel frame clad externally with 6 mm thick polycarbonate on the front (door) and left- and right-hand sides. Although expensive, polycarbonate is strong and does not split like Perspex when hit or drilled. The visibility provides some sense of security for the animal.
- There is typically a 30-40cm solid metal skirt around the bottom of the headbox, which includes the door and all other 3 sides. The floor and roof are normally not clear but can be any plastic, steel or strong wood. Remember the floor will get wet.

1.2.2 Front:

- The door is typically at the front of the headbox, with feed and water accessed through it.
- The door can be the full height of the headbox or only opening the lower portion (see Indonesia example file), to allow feed to be put in and removed.
- The door should be sealed by 2-3 'toggle-latches', which enable the tension to be changed independently. A good seal on the door is desired but not essential.
- The inside-face of the door can be fitted with adhesive foam strip (as used to weatherproof windows) to make the door seal onto the headbox near-airtight.

1.2.3 Back:

- The back of the headbox, where the animal stands, should have space for them to lie down and rest. Design suggestions: 'V' frame, curved or cut away section, stanchion-like, adjustable entrance.
- The animal enters from the rear through the hood and is tethered there by various means. (see 1.2.5)



1.2.4 Hood:

- Designing the hood (or shroud) is always difficult. Ultimately a 'funnel' shape is needed, long enough to allow the animal to reach in for feed, and to pull back to lie down.
- Design: Details depend on the design and dimensions of the back of the headbox. Experiment with cheap fabric/plastic first and keep the final template for making replacements in future years. It is also good to have an extra hood available for replacement in case the hood gets damaged.
- Fabric: Needs to be air- and water-tight. Must be flexible and strong, not torn easily, or be prone to cracking. Sewing is recommended.
  Vinyl coated polyester (as used in car upholstery) is good. Coated canvas may be available but cracking may be a problem long term. Lighter material (possibly tent fabric) adds less weight stress to the neck of the animal but may not be air-tight.
  Having some clear panels, or even the complete hood, will help the animal not to feel isolated.
- Adjustment: A drawstring rope adjusts to fit around the neck once the animal is in place. There must be a space around the neck, loose enough to fit 2 fingers (2-5cm) between the neck and hood. The intention here is that the fit around the neck be LOOSE, as this is the only location that air can be drawn to the hood from. So it is important to not seal off around the neck or no air will enter the headbox.

1.2.5 Tethering:

- Some animals are accustomed to being tethered and can be tied to the bar across the front of the headbox. (i.e. RUA uses halter with nose ring and tether in individual pens, long-term which trains animals).
- For others, a chain fixed at the top and of the rear entrance, with a sliding collar, is recommended. "D" shackles allow adjustment in the length of chain, so that animals can both access feed and withdraw far enough to lie down. Covering the chain, possibly with plastic hosing or other flexible pipe, allows the collar to slide freely up and down the chain as the animal moves, so that the sliding collar does not get caught on the metal chain links (see Laos NUoL example)
- The collar can simply be a circle of covered chain, which slides up and down as needed.
- For animals not accustomed to being tethered, training before the experimental period is essential.

1.3 Services:

1.3.1 Electricity:

- Have a generator as a back-up for when electricity supply fails. Generator must be large enough to power the whole set-up, with the wiring and plugs to connect to. This option should be put in only if the right wiring and system is available, e.g. 3-way breaker.
- Apart from the access door for feeding, a safety door may also be installed (discussed 7.1), to ensure animals do not suffocate if either the electricity shuts down or the exhaust fan fails for some other reason.

1.3.2 Water:

- Water should be continuously available.
- The water supply should be positioned so that water spillage or splashing by animals does not wet feed in the feed bin. A small hole in the floor can drain any spilt water.
- The hose/pipe providing the water must be protected or unchewable by the animal when the animal is bored, to avoid damage or leakage.
- Ideally the water supply should have a low-pressure water source & be supplied through a water dish with a float valve stopping overflow. A header tank can help avoid high-pressure blowout problems.

- Position the water dish so it's not directly above or too close to the feed bin. A splash guard/drip catcher may also be used.

1.3.3 Feed:

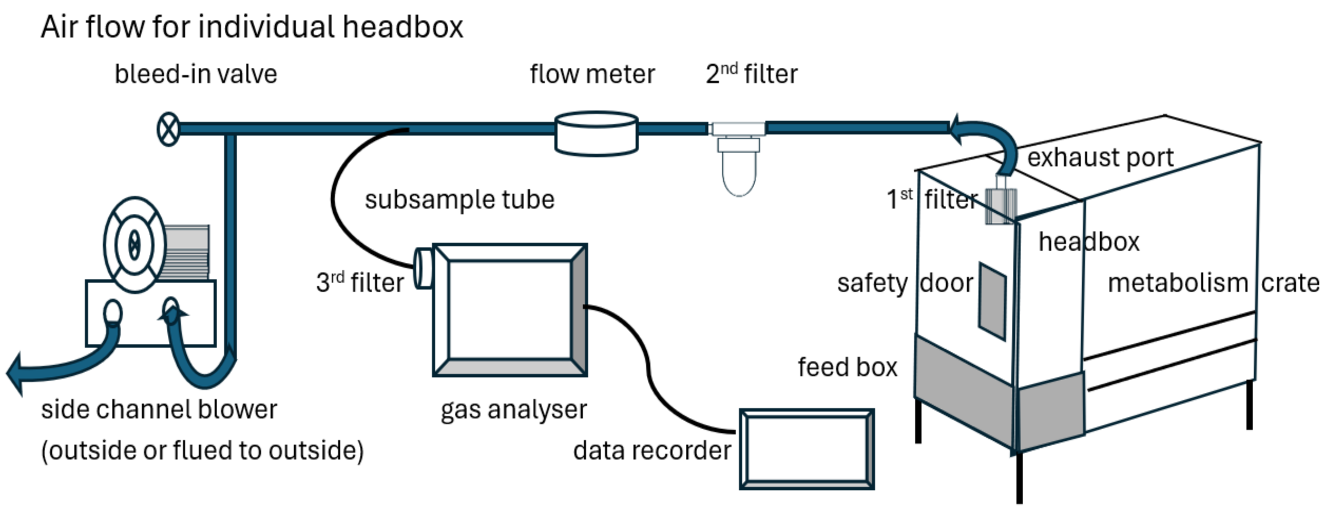
- Feed bins are not essential but are helpful for weighing, and feeding rations and weighing refusals
- Typically, the sides of the headbox around the feed bin are tin not plastic, but this is not critical.
- Subject to the type of feed, the volume of feed may be more than can be fitted in the feed trough at once, requiring opening of the door to add feed 1-2 times/d after the door is shut. In continuous gas collection systems (Tedlar bags), the small loss of gas across the whole day is negligible. In multiplexed systems with cyclical sampling, it is best to not include data from the time period when the doors were opened.

1.4 Air Flow: Components:

1.4.1 Blowers to draw air through the hood:

The usual components of the airflow and measurement system and their order of occurrence are shown below (drawing air from right to left in the diagram):

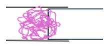
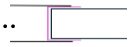
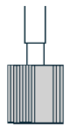
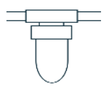
- At the start of the air path fresh air is drawn into the headbox from around the neck by negative pressure (vacuum) from a an air blower placed at the very far end of the air flow path
- Almost universally, ambient air is drawn through the headbox system using side-channel blowers (SCB, also called ring blowers).
- SCBs are not expensive so use 1 blower per headbox so each headbox has its own independent airstream. This reduces animal health risk in the event of mechanical failure of a SCB.
- Use SCBs that are more powerful than you need and are capable of drawing more air than needed through the headbox. This gives you extra opportunities to increase air flow if you have problems with high humidity and condensation.
- To control the flow rate, install a 'T' piece between the SCB and the flow meter with a gate valve. You can use ball valves but the ability to regulate flow exactly is not so fine as with a gate valve. Opening the valve fitted on the side arm will draw air in from the environment and reduce the flow of air to the SCB from the headbox.
- A variable speed drive (e.g. Teko) can also be used to regulate the flow rate, but this is as expensive as a new SCB and only allows perhaps 15% change in air flow.



1.4.2 Ducting:

- \- The air is drawn out from the headbox via a manifold (PVC pipe 5 cm diameter for cattle) which fits to the exhaust port in the roof of the headbox. This can be either a ‘T’ across the top or an inverted ‘U’ coming down the sides as well. It is perforated with many holes to allow air entry and to ensure the air is drawn from throughout the headbox.
  \- The manifold must be cleaned periodically (between experiments is best) to prevent blocking.
  \- To ensure linear flow of air past the flow meter, some straight 5 cm-diameter ducting (pipes) is needed both sides of the flow meter. Typically this straight pipe must be 10 times the diameter of the pipe on the inlet side and 3 times the diameter of the pipe on the outlet side of the meter (photo below). Some flow meters come with this fixed pipe manufactured as part of the flow meter. Beyond this, flexible ducting can used if necessary. The lengths of straight pipe needed can be found in the Specifications of your flow meter.
  \- Always carefully seal all open ends of your ducting when not in use, to keep out dust and critters.

1.4.3 Filters:
Air must be filtered before it gets to the flow meter, and also before entry to the gas analyser.
There are many sorts of air filter, primarily to remove particulates (feed dust, hair etc):

- 1st filter: a coarse filter is put at the exhaust port to prevent dust and feed-trash entering the system.
  A wad of air-conditioning filter can be inserted across the exhaust port entry, or... 
  a stocking can be placed/jammed between the manifold and the exhaust port, or... 
  a car/bike air intake filter (e.g. with fins) can be fitted directly onto the exhaust port, replacing the manifold, or onto either end of the manifold. The manifold is not perforated if this is used. 
- 2nd filter: in-line filter for finer particulates and water removal is placed between the headbox and the air flow meter.
  In-line filters with a ‘bowl’ water trap, such as SMC brand filters are often used. These are designed for positive pressure and may not seal when used when the pressure inside the pipe is lower than atmospheric pressure, i.e. the vacuum created within the headbox system.
  NB: Always check for complete seal. 
- 3rd filter: a small volume membrane filter can be added just before the gas analyser.
- Make sure all filters do not leak. Check regularly.

1.4.4 Other considerations:

- **Circulating Fan:** An oscillating air circulation fan is mounted inside the headbox to homogenise the gas composition within the headbox and prevent layering of expelled gasses.

- **Dehumidifier:** On fresh forage diets and/or in highly humid environments, a portable household dehumidifier (~USD$30) can be placed in the HB to remove some of the moisture, with wastewater draining outside the headbox. The addition of dehumidifiers theoretically maintained the air flow rate in the pipe, whereas increase without the dehumidifier was observed to be about 20% lower (UPLB). Mechanism is unclear.
  - Humidity/Temperature: Installing a dehumidifier and humidity/temp sensor with a digital display is useful (~USD10)
  - Humidity can also be reduced by increasing the airflow, diluting ut the water in cow breath but this also reduces the ppm of CH4 and CO2 in the measured samples

1.4.5 Safety Doors:

- The principal animal welfare risk in confining animals in headboxes, or any respiration system, is the risk of suffocation if airflow is lost. This may result from: (a) power failure stopping the flow of air through the headbox, or (b) mechanical failure of an exhaust fan or blockage of the airflow system, stopping removal of air from the headbox.
- Safety can be improved by:
  - Using an UPS (uninterrupted power supply). These are expensive and offer limited support.
  - Having students sleep nearby and get awoken by (a) a light that comes on when the power stops, (b) a telephone message being sent when power goes out (in like manner to a freezer alarm), or (c) a battery powered alarm which activates when electricity stops. These approaches may also not be foolproof.
  - A generator is needed to provide backup power, with the wiring and plugs to connect to.
  - None of these will protect an animal if a fan seizes while the electricity remains on. It is better to have an automated safety system that does not require human response and intervention to assure animal safety.
  - The safest system is achieved by installing a safety door that opens automatically in the event of (a) a power failure or (b) CO_2 accumulation in the headbox.

- Safety doors need to be plugged in to the power supply and open outwards under gravity when the electro-magnet releases during a power failure.
  - They are best installed low in the headbox (to provide air closest to the animal) but where they are not obstructing workers.
  - Safety doors will remain open when not in use. Tie them shut tightly and cover to protect the sensor and magnet box. **NB: MUST UNTIE TO USE!**
  - Discard data from the period if doors have opened, due to electricity outage. For prolonged outages, discard data for the whole day and repeat.

1.4.6 Gas sample collection:

- The gas subsampling tube must be placed after the flow meter but before the bleed-in valve(that may be allowing atmospheric air into the air duct between the flow meter and the SCB as means of setting the flow rate from the HB at the specified level.
- Always try and keep the distance between the sample point and the gas collection bag or analyser as short as possible & narrow bore (eg 3mm dia). If gas is being collected continuously through a peristaltic pump into a bag, then the most gas impermeable bags are Tedlar, and a 4L or 5L Tedlar bag can be used to collect 2-3ml/min of sample over the 22-24h collection period.
- If the sample is being drawn directly into a gas analyser then flow rate will be higher so a wider diameter tube (up to 6mm diameter) is appropriate.

1.4.7 Gas Analyser:

- The brand of gas analyser is not important. Whatever analyser you use be sure to:
  - Read a ‘zero’ gas sample (usually pure nitrogen N2, industrial N2 is fine) & record there adding (hopefully 0 ppm CH4).
  - Read a span gas sample (a standard analytical gas whose CH4 ppm is close but higher than your expected ppm. Often 1000ppm is used)
  - You may want to use the zero and span gases to <u>recalibrate</u> your analyser
  - If you do not recalibrate then you can simply create a regression between measured ppm (eg. 2 ppm and 990ppm) and expected/certified ppm in the cylinders (eg 0 and 1000ppm) for the zero gas and span gas. Then use this regression to adjust your measured sample ppm values.
  - Once you are confident in your analyser & the readings of the known gases
    - If you are using gas collection bags then measure your background sample collected from inside the animal house near where the air is drawn into the shrouds. This is the MOST important sample you will subtract this value from the values measured inside the HBs
    - Measure each of your samples from the headbox (typically 1 bulked sample/d/headbox)
    - Remeasure your ‘zero’ and span’ gas concentrations and record these.

    - If you have a multiplexer drawing samples from each HB AND from the ambient air in the animal house, check your analyser against the zero and span gases each day. As described above, if you don’t recalibrate before you start you can adjust your readings based on measured v expected ppm regression as explained above.

2. Headbox Operation:

2.1 Air Flow:

There is a trade-off between low flow rates to give you high gas concentrations (but also high humidity and temperature) and high flow rates which give lower gas concentrations (but which reduce risk of condensation and give more scope for evaporative cooling).

- The ability of your gas analyser to differentiate gas concentration at low ppm is a key consideration when setting your flow rates. If the sensitivity of your analyser is poor, use a lower flow rate to ensure high ppm CH4 are being measured.
- Higher gas concentrations help to differentiate headbox data from background levels, although the total gas produced over time will be the same.
- While cattle in large (20m^3) open circuit respiration chamber facilities use airflows of 3-5 m^3/min, the rates used in cattle headboxes where the total HB internal volume is <1.5m^3 is much lower and typically 400-600L/min (0.4-0.6m^3/min) for cattle and 120-200L/min (0.12-0.2 m^3/min) for small ruminants.
- Some people calculate flow rates according to the body weight of each animal, using higher flow rates for larger animals. This may reduce [CO2] variation between headboxes, to optimise within the range of the analyser. It also may help maintain [CO2] below 1%, to avoid physiological impacts of higher [CO2]. It is not essential to vary flow rate for animal bodyweight.
- Higher flow rates may also help to manage temperature, humidity, and condensation levels in headboxes housing larger animals.

2.1.1 Measuring Air Flow:

There are many suitable air flow measurement systems used with headboxes. The main ones in increasing order of complexity are:

- Dry gas meters: These are the boxes usually used by domestic gas companies to measure combustible gas flow to your house if piped directly in. They are fully manual and rely on bellows of known volume being filled and emptied and the total flow is tallied. NO electricity is needed. ~ USD$1100 for cattle size. NO built-in temperature or pressure correction, so you need to record and correct manually for your conditions.
- Turbine gas meters: The passing air rotates a turbine (similar to that in a jet engine) and each turn of the turbine equates to a known flow though the meter. ~$1200 for cattle size. NO built-in temperature or pressure correction, so you need to record and correct manually for your conditions.
- Mass flow meter: Normally this is a 'thermal dispersion' type, where the temperature differential between 2 pins correlates with the flow of air passing between them. ~High cost for cattle size. MFMs are ALWAYS temperature and pressure corrected and report the flow at some specified temp and pressure, but NOT at the ambient conditions. You need to look at meter documentation to know at what temperature and pressure it is reporting flow, and correct the flow to standard temperature and pressure (STP: 0°C, 101.325 kPa)
- It is very important to make sure that feed particles do not get trapped in the flow meter and distort it. A good indication of this problem is if one flow meter suddenly starts to show lower or higher flows than it has before, when the valves have not been adjusted. If you see this take the flow meter out and clean it CAREFULLY. To prevent this issue, use good filters, as discussed previously (1.4.3).
- If a flow meter measurement is persistently lower/different than the others, there may be a flow meter problem which needs to be found and fixed.

2.1.2 Allowing for moisture in air flow:

- We measure flow of air as the air comes from the headbox, but in almost all cases, the gas analyser or preparatory analysis steps DRY the air. The air is dried for 2 reasons, to (a) prevent condensation inside the instrument, and to (b) avoid water interference at the wavelength used to measure methane concentration in an infrared cell.
- Water vapour is always less than 5% of total air volume, so it is not a major correction. Ideally though, we would reduce the recorded flow by this percentage to convert our <u>measured wet-air airflow rate</u> (e.g. 500L/min as measured to a <u>dry-air flow rate</u> that may be 1-4% less).
- The percentage of water able to be held increases as the air temperature goes up. This can be calculated using Teten's equation with knowledge of temperature and relative humidity of the air where flow is measured.
- The equation to calculate the % water vapour held in air is detailed in Section 4.3.3

2.1.3 Allowing for Temperature and Pressure:

As described above (2.2), some flow meters do not account for temperature and pressure. Since we rely on knowing the temperature and pressure of a gas to convert from volume to mass (using the molar volume of an ideal gas), we MUST measure temperature and pressure.

- Without temperature and pressure data to connect with, the volume of methane produced is a meaningless number.
- In most systems the drop in pressure through the airpath from a headbox to a side channel blower is less than 5kPa (or less than 5% error if not accounted for), but it is worth correcting for. To do this we measure the temperature and pressure inside the ducting, between the headbox and the flow meter.

- At RUA this is done using a hand-held probe that is placed temporarily into the sampling port near the flow meter. The probe measures all necessary data: Temperature, Pressure, and Relative Humidity (%).
- This can allow us to calculate dry-airflow precisely from the total-airflow, using the Equation below:
- Equation to convert total-airflow to dry-airflow: (see 4.3.2)

2.1.4 Humidity control of headbox air:

Wet forage and animal breath and eructation are great sources of water vapour. In headboxes in humid environments it is VERY EASY to get condensation in the headbox. This is not necessarily a problem, but it is unattractive, indicates a very humid (& usually hot environment) for the animal, and importantly, it creates a major risk of water condensing in the flow meter or analyser. To minimise the risk of condensation:

- Increase air flow through headbox.
- Install a portable house-hold dehumidifier.
- To minimise the risk of condensation in flow meter or analyser, keep air warm at these two places, possibly by avoiding air-conditioned cooling near the analyser.

2.2. Gas Recovery:

2.2.1 Gas recovery tests:

For quality assurance, we need to perform gas recovery tests, preferably both before and after using the headboxes in an experiment. Any variations in gas recovery can then be corrected for in your collected data.

To be sure that your estimates of daily methane production are correct, it is essential that you measure and allow for the recovery of a known amount of gas which is entered into the system. This is true for open circuit respiration chambers as well as headbox systems, and the same procedures are used. Options include:

- Burning propane in the headbox and measuring CO_2 produced and O_2 loss (ETH) – seldom used.
- Burning steel wool (Bruce Young) – seldom used.
- Releasing methane at known rate (UNE).
- Releasing CO_2 at known rate (RUA).

Gas can be released either directly to the exhaust port or released and mixed into the headbox space. These two most common systems for ASEAN headboxes are described below. The choice is often determined by the accuracy of your gas analyser and the availability and cost of gases.

2.2.1.1 Direct infusion to exhaust port:

- Releasing directly up the gas intake pipe is quick, simple, and precise, but may not identify gas losses from the whole headbox.
- Because the introduced gas is passed directly into the exhaust outlet and does not mix with the 1-1.5m^3 air in the HB, it very quickly (within seconds). Consequently, it is really a test to check whether the analyse and flow meter are working correctly and rightly estimate the amount of gas you have infused into the gas stream.

2.2.1.2 Infusion to whole chamber:

- This is a slower procedure but if the recovery value is correct (95-105%) the operator can be confident that no gas released by the animal is being lost by 'leakage' from the system and that the analyser and flow meter are working correctly.

- It relies on the logarithmic rise in marker gas (CO_2, CH_4) that is being introduced over time at a constant rate (eg 100ml CH_4/min) into a headbox that has air being drawn through it at a constant rate (eg 500L/min).
- The ppm of the marker gas will rise logarithmically in the HB and its exhaust, and over time will reach a plateau (fixed maximum) equilibrium value We can then use the same procedure to estimate whether this rise in ppm of marker gas is 80,90, 100 or 1010 of what may be expected from the amount released and the volume of air entering the chamber.
- Whole chamber release uses more gas and time (to achieve a plateau concentration) but can identify whole system issues.
- Lower recovery when putting it in the chamber than to the exhaust directly

2.2.2 Recovery of added CO2:

2.2.2.1 Release directly into air outlet pipe (CO2):

- In this approach, CO_2 is released from a small cylinder that is sitting on a balance able to weigh to at least 1g accuracy. The flow from the cylinder is set (crudely) using a rotameter attached to the gas regulator. A gas analyser is connected to the sampling port on the exhaust gas stream so that [CO_2] in the passing exhaust gas can be instantly recorded when required. The flow is set at 5L/min from the CO_2 regulator with the rotameter adjustment. A tube is used to direct the gas from the regulator into the headbox and right into the exhaust duct. As the cylinder is opened at the main valve, the weight of the cylinder is recorded every 30 seconds for 3 minutes, and at each 30s occurrence the [CH_4] is also recorded.
- When using CO_2, CO_2 release is commonly set at 5L/min from the CO_2 regulator with the rotameter adjustment. A tube is used to direct the gas from the regulator directly into the headbox, away from the exhaust duct. Gas is released at this rate, equating to 10g CO_2/min, for 20 minutes. Weight of the cylinder and [CO_2] is recorded every 5 mins. The recovery is taken from the plateau of [CO_2].

A regression is done to estimate the rate of release of CO_2 from the cylinder (g CO_2/min). Data on the average [CO_2] in the exhaust air stream over this period is coupled with the average flow rate through the headbox (usually based on total flow over 2-3h at least) together with temperature and pressure. Together this data allows calculation of what the [CO_2] should be if all the CO_2 released passed through the exhaust.

The recovery is then calculated, using data adjusted to STP as:

= (measured rise in [CO_2] due to CO_2 release/ expected rise in [CO_2]) * 100

2.2.2.2 Infusion to whole chamber (CO2):

If your analyser only expresses CO_2 in % (typically down to 100ppm or 0.01%), then you will need so much CO_2 in order to be confident in the reading that using CO_2 for long term infusion becomes impossible. So if you want to infuse CO_2 for along period (20-30min) you need to have a gas analyser that reads to 1ppm. If you have this proceed as for CH_4 infusion below.

2.2.3 Recovery of added Methane CH4:

The advantage of using CH_4 not CO_2 for recovery tests is that analysers generally have a lower detection limit for CH_4 and so much less is required to undertake a recovery test.

Either short term (2.2.2.) or longer term (2.2.2.2) introductions of methane can be made as for CO_2. A rate of CH_4 release of approximately 100ml CH_4/min is generally used with either system. The release rate can be higher or lower but it MUST be exactly known, and for this reason mass flow controllers are usually used to release the gas at a constant rate.

2.2.4 Calculations:

The data collection sheet and recovery calculations are provided in excel workbooks associated with his report. As indicated below.

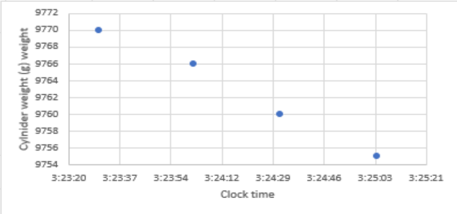
Cambodia Gravimetric Recovery test

Insert real numbers in the yellow boxes of the template below. Recovery % is calculated in bottom right

Date	27/05/2024		
Headbox	1		
Temperature (oC)	32.6		
Pressure (hPa)	990.9		
Determining headbox flow rate		Time	Flow readingM3
START of air flow Monitoring		2:05:10	1245
END of air flow Monitoring		3:05:00	1275
	Time	Cylinder weight (g)	Co2 (%) measured
Before CO2 gas turned on	3:20pm	9805	0.045
Check that the CO2 is being piped all the way into the headbox exhaust and that the gas analyser is warmed up and drawing gas from the sample port on the correct headbox gas line			
Time gas was turned on	3:23:00	9775	X
Set outlet flow to approximately **5L CO2/min**			
After 30s	3:23:30	9770	1.05
After 1 min	3:24:02	9766	1.06
After 1.5 min	3:24:31	9760	1.06
After 2 min	3:25:04	9755	1.05

Note: it is good to get at least 30min of air flow rate data to allow air flow to be estimated correctly

	litres	minutes	Litres/min
Flow rate as measured =	30000	59.83	501.39
Flow rate at STP (0oC, 101.327kPa) =			438.03



Use the slope of this weight v time graph to estimate the rate of weight loss of the CO2 cylinder

Rate of release of CO2 at ambient conditic -9.82382 g CO2/min

Now need to convert this grams of CO2 to litres, & need to make this conversion @ STP (0oC, 1 atm pressure)

Since 44 g of CO2 occupies 22.4 L @ STP, the volume release at STP woulc 5.0012 L CO2/min at STP

3. Animal Management

3.1 Animals:

3.1.1 Animal numbers and statistical power of experiment:

It is essential to design your experiment to maximise statistical power within the physical and financial constraints of your situation. Use the link below to compare your options.

https://samplesizecalculator.ucdavis.edu/

Sample Size Calculator

The database contains **32** reports for this combination of number of treatments, methane technique, and experimental design.

The CH_4 yield and its variability (SD) are average values from the database. You can customize these values.

Methane Technique	Experimental Design	Methane Yield (g CH_4/kg DMI)	Alpha (Type I Error Rate, α)	Effect size, $f = \sqrt{\frac{\sum_{i=1}^{k}(\bar{x}_i - \bar{X})^2}{k}}$/pooled SD
Respirometry chamber	Latin Square	20.8	0.05	0.4094
	Number of Treatments	Expected CH_4 Yield Reduction (%)	Power (1 - β)	Sample Size per treatment
	4	10 10 10	0.8	12
	Number of Periods	Pooled SD of CH_4 Yield	Correlation (ρ)	Total Sample Size
	4	2.2	0.5	12
				RUN

3.1.2 Choice of statistical design:

In general, **Latin-square** experiments use fewer animals but take much longer to complete.
Fewer animals are easier and cheaper to source and can be better matched for less variation.
There is a higher risk of issues arising (sourcing consistent feed supply, animal health, staffing, etc.).
Conversely, **Randomised Complete Block Design** experiments generally can be completed more quickly but require more animals. A covariate period of at least a week is needed with all animals on a standard diet, before animals go on treatment diets.

There are many other factors that will constrain your choice of statistical design, and therefore your experimental protocol. Have this sorted as your first priority.

3.1.3 Choice of animal:

It is preferable to have matching animals, but this is not always easily achieved.
Do not mix sexes, if at all possible (i.e. bulls vs cows)
Try to not mix physiological state (i.e. milking vs non-milking)
Try to match age, especially if you are looking at growth or production (i.e. weaner heifer vs mature cow)
Try to match body weight.
Randomised Complete Block Design uses blocks to overcome high variation in animals.
Latin-square overcomes variation by using individuals as their own controls.

3.2 Housing:

3.2.1 Restraint in headbox: Options include:

- chains with sliding collars
- halters with tethers tied to front of headbox
- sliding bars behind to prevent backward movement

3.2.2 Flooring and structure: Best practice includes:

- Rubber flooring and sides
- Nothing protruding to catch or cut animals

3.3 Feeding:

3.3.1 Dietary adaption:

Animals must be adapted to their treatment diets for long enough to reach a steady-state in their microbiome.

- It is best to introduce new diets gradually, preferably over 7 days.
- Animals MUST be fully on their experimental diet for 14 d at least before methane is measured and Ym calculated. Preferably have animals on diet for 21d before sampling (although this may include the introductory period).
- If animals are in a Latin-square experiment a 'wash out period' of at least a week (with all animals on a standard diet) should be incorporated before each feeding period.
- If a Latin square is not used, then a covariate period before the study should be used to allow for individual animal variation in Ym and to increase the probability for detecting treatment effects.

3.3.2 Managing animal feeding:

The CH4 measured today is produced from the feed eaten today (%) + the feed eaten yesterday (%) with 15% of the variation in todays feed intake being explained by yesterday's feed intake Robinson, et al. (2011). "Factors affecting variability in feed intake of sheep with ad-libitum access to feed and the relationship with daily methane production." In Proceedings of the Association of Advancement of Animal Breeding and Genetics, vol. 19, pp. 159-162. 2011). Therefore, it is essential to have precise DMI data for the days preceding measurement, and if at all possible, to have a constant DMI over these days. Otherwise you will need to make some estimation of the weighted DMI over the day of measurement and on the preceding day.

3.3.3 Dry Matter Intake:
It is ESSENTIAL that the daily DMI is known for any day of methane measurement, but also the intake on the preceding day or 2 days. This requires sub-sampling and Dry Matter calculation, of both feed and refusals, around the CH4 measurement period. It is also VERY helpful to measure Gross Energy content of feed and refusals to enable calculation of Restricted Ym (% of GE intake lost as methane, in gCH4/d). Often for inventory work and IPCC this accurate Ym can then be applied to the animal intake and performance over a longer period when animal eating *adlib*.

Organize your feeding in a DM basis; TMR feeding allows for easier DMI calculations

3.3.4 Off-feed in the Headbox and restricted feeding:
Inevitably, some animals may reduce their ad-libitum DMI when put into the headbox, reducing the accuracy and value of their results.

- This drop in intake (see Robinson et al paper cited above) may not arise if animals are regularly housed in the headbox and crate every day of the experiment, but this approach may not be possible in your situation.
- It is recommended to restrict feed offered for the days before and during headbox measurement, in the following way: Feed all diets *adlib* and record DMI for the first 14-21 days. Calculate average DMI and set feed offered at 80-90% of average *ad-lib* DMI. Offer the restricted diet for 7 days before and including time in the headboxes.
- Feeding a restricted ration ensures that refusals are minimum and Ym can be accurately calculated.
- There may be complications (i.e. animals being hungry, bored, and problematic) and limitations (i.e. dairy cows cannot ethically be restricted below 90% of ad-lib ration). But for the best data, it is best to follow to this protocol. The exact Ym achieved can then be scaled-up to the remainder of the experiment where DMI maybe ad-libitum by multiplying Ym (g CH4/kg DMI) by kg DMI/d and to calculate daily emissions emissions/kg animal product

3.4 Animal Handling:

3.4.1 Introducing animals to headboxes:

- Most animals are shy on entry to the headbox, so the following is recommended. To lead the animal into the HB it generally has a head- or nose-halter so it can be led, and the lead-rope is passed through the shroud from behind and used to lead the animal into the shroud and secure it there by one of the means above (Section 3.2.1)
- Animals are usually introduced over a period of at least a week. With animals being brought in for an initial 3h visit before returning to their pen, then a 6h visit , 9h visit and finally several 24h visits. Soe ach day of few days ana animal is led though this process so it is comfortable with the feeding and human interaction and physical restraint of being in a headbox
-

3.4.2 Cattle with horns:

- Choose polled cattle whenever possible. If cattle must be dehorned, allow for a long recovery before the experiment. Using young cattle with immature horns is another option.

4. Administration

4.1 Animal Welfare:

4.1.1 Animal Ethics Approval:
If your country and Institution has an active Animal Ethics approval system, you must use this. Prepare and submit your proposed research well before you start to allow for appraisal time.
Abide by all requirements, including all documentation.

Some countries do not have an active Animal Ethics (AE)approval system. It may not be possible to access Animal Ethics approval through another country, but this is worth exploring. Publishing research from animal experiments that have not been approved by an animal ethics committee is getting very difficult!!!!!!!!!!!
As far as possible, follow Best Practise as outlined in AE requirements from another country, including all requirements and documentation.

4.1.2 Eligibility to publish:

Many Journals require research to be conducted with Animal Ethics Committee approval. Not having AEC approval will limit where your paper can be published.

The paper by Ssuna et al (2024) listed below has recommendations to follow but may still not satisfy journals who require AE committee approval.

Ssuna, P., Crump, A. and Siegmund, K., 2024. Animal Welfare Guidelines for International Development Organisations in the Global South. *Animals*, *14*(13), p.2012.

4.1.3 Incident Report:

If something goes wrong, record everything.

Write up an Incident Report. Describe the events leading up to the incident. Include everything you did to mitigate the problem. For example, how you responded and what actions you took, who you rang for advice, what treatments you gave, etc. Include how you adjusted the remaining animals and experimental procedures.

-

4.3 Corrections to data

In the above discussion, we have identified a number of checks and constraints to measurement that may required to be accounted for by adjusting the final data. These are described below

4.3.1 Correct for Temp, Pressure,

Gas analysers report gas concentration in parts per million, which is a volumetric or molar ratio parameter. If the sample has 100ppm CH_4 in air, then there is 1 unit of volume CH_4 per million volumes of total gas. To be able to convert volume (ml) to grams of methane, we need to account for the temperature and pressure when the concentration is determined.

The universal gas law is then used to convert the volume of methane as measured (= ppm x Vol of air flow through the HB/d) to the volume at Standard temperature and pressure (STP) as below

The most common way to express the Combined Gas Law is:

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2}$$

Where:

- P_1, V_1, and T_1 represent the initial pressure, volume, and absolute temperature of the gas.
- P_2, V_2, and T_2 represent the final pressure, volume, and absolute temperature of the gas.

We know that one mole of any ideal gas (& we assume we are dealing with ideal gases), occupies 22.4 L at STP.

Hence for methane, 1 mole weights 16.4g and occupies 22.4L at STP. We sue this to convert our litres of CH4 at STP to g CH4.

4.3.2 Correct for Humidity

We know that water can occupy up to 5% of the total volume of air when saturated (100% Relative humidity) and that our flow meters measure 'wet air flow' , but our analyser typically dry the gas before measuring ppm, because vapour can interfere with CH4 determination at some wavelengths.

So while water vapour is usually only 2-3 % of air volume, we can correct our measured 'wet air flow rate' to a 'dry air flow rate' if we know temperature, pressure and relative humidity as shown below using partial pressures. The % of water volume (Step 3 below) can then be removed from the wet air flow rate as determined by the flow meter.

To calculate **what percentage of air volume is water vapor** at a given temperature and relative humidity, you can use the following equation based on **partial pressures** and the **ideal gas law**.

Equation:

$$\text{Water Vapor Volume \%} = \left(\frac{p_v}{p_{total}}\right) \times 100$$

Where:

- p_v = actual **partial pressure of water vapor** (in Pa)
- p_{total} = total atmospheric pressure (typically 101325 Pa at sea level)

Step-by-Step:

1. **Find saturation vapor pressure** $p_{sat}(T)$ at a given temperature T (°C).
   - Use the **Tetens formula** (valid from -40°C to 50°C):

$$p_{sat}(T) = 610.94 \times \exp\left(\frac{17.625 \times T}{T + 243.04}\right)$$

- Output is in Pascals (Pa).

2. **Calculate actual vapor pressure** p_v using relative humidity:

$$p_v = RH \times p_{sat}(T)$$

- RH = relative humidity (as a **fraction**, e.g., 60% = 0.6)

3. **Plug into volume % equation:**

$$\text{Water Vapor Volume \%} = \left(\frac{p_v}{101325}\right) \times 100$$

4.3.3 Correct for Gas Recovery %

Gas recovery can be expected to be between 95 and 105% of that infused. Some laboratories accept hat their analytical system itself has at least 5% error so do not adjust the final data, but some labs on-principle, correct their experimental gas measurement by the recovery through the headbox & this is to be recommended. While leakage from the system can explain recoveries of added gas less than 100%, there is no reason other than incorrect flow or ppm measurements that can explain a recovery greater than 100%. For this reason, correction of data for recovery is encouraged.

4.2.4 Correction for flatus

Our general desire is to determine the total daily enteric methane prosecution from an animal. We recognise that much of the hind-gut generated CH_4 is voided via the blood stream and breath, but there is loss in flatus that is not determined by the headbox system. There is very little literature on this (Principally Murray, R.M., Bryant, A.M. and Leng, R.A., 1976. Rates of production of methane in the rumen and large intestine of sheep. British journal of nutrition, 36(1), pp.1-14.). It can be expected to vary with forage type (based on differential foregut/hindgut digestion from starch verses fibrous diet components) but increasing the measured cH_4 emissions from headboxes by 2-5% would seem appropriate when reporting total daily methane production of ruminants.

4.2.4 Correcting or managing for feed intake variation

Adapting animals to the headbox so that their feed intake does not vary during the measurement period is fundamental to useful measurements of gas production. It is to avoid day to day variations intake that fixing intake at 90% of previous ad-libitum levels was recommended. The study in sheep of Robinson, et al (2014) *"Sire and liveweight affect feed intake and methane emissions of sheep confined in respiration chambers."* Animal. 8: 1935–1944. Found the effect of DM intake on daily sheep emissions was 50.9% attributable to the intake on the day of methane measurement, 34.2% attributable to feed intake the previous day and 14.8% attributable to the feed intake 2 days prior to CH_4 measurement. So it is clearly important to either stabilise daily feed intake prior to methane measurement or allow for it using a weighted estimate of feed intake over the day of measurement and at least the day before.