BEST PRACTICE GUIDELINES FOR HEADBOX USE TO MEASURE GREENHOUSE GAS EMISSIONS FROM LIVESTOCK

Contents

BEST PRACTICE GUIDELINES FOR HEADBOX USE TO MEASURE GREENHOUSE GAS EMISSIONS FROM LIVESTOCK

Introduction

Accurately measuring greenhouse gas (GHG) emissions from ruminant livestock is critical, especially in regions like Southeast Asia, where agriculture is key to both economic and environmental sustainability. As climate-smart farming practices gain attention, reliable and region-specific methods for quantifying methane emissions are essential.

The respiratory headbox system is a cornerstone of livestock GHG research, based on its cost efficiency, simplicity, and minimal need for additional equipment beyond gas analyzers. It has become a vital tool for assessing methane emissions from different regional diets and testing mitigation strategies. However, precise and consistent measurements demand using respiration headboxes with strict quality assurance protocols and standardized operating procedures.

Since the headbox is the primary equipment for measuring GHG emissions, proper operation and troubleshooting are crucial. Users must be well-trained in its functionality to maintain data accuracy and reliability. This written guide provides best practice guidelines to ensure optimal headbox performance, helping researchers obtain dependable emissions data for effective climate mitigation in livestock production.

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.

1.2 Design, dimensions & materials:

1.2.1 Headbox:

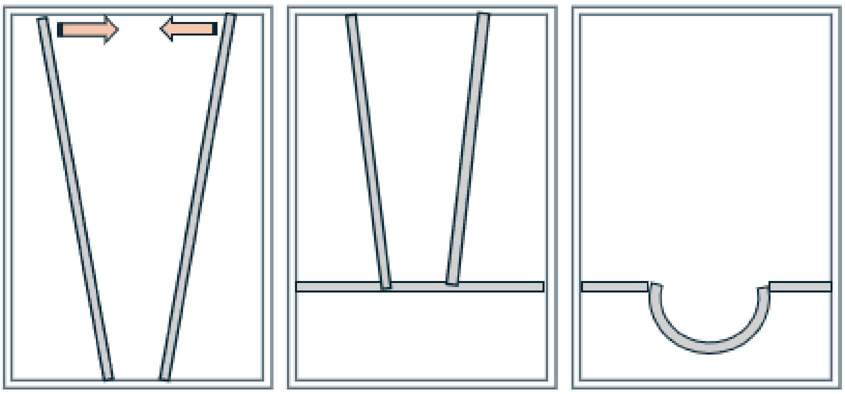
- For large ruminants, the headbox should be approximately 80-100cm Wide, 150-175cm High, 75-90cm Deep, and may be built straight onto the ground or raised from the ground by 25-30cm. For smaller cattle (such as yellow or Bali cattle, a smaller headbox is appropriate).
- For small ruminants, the headbox should be approximately 50-60cm Wide, 75-100cm High, 40-60cm 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:

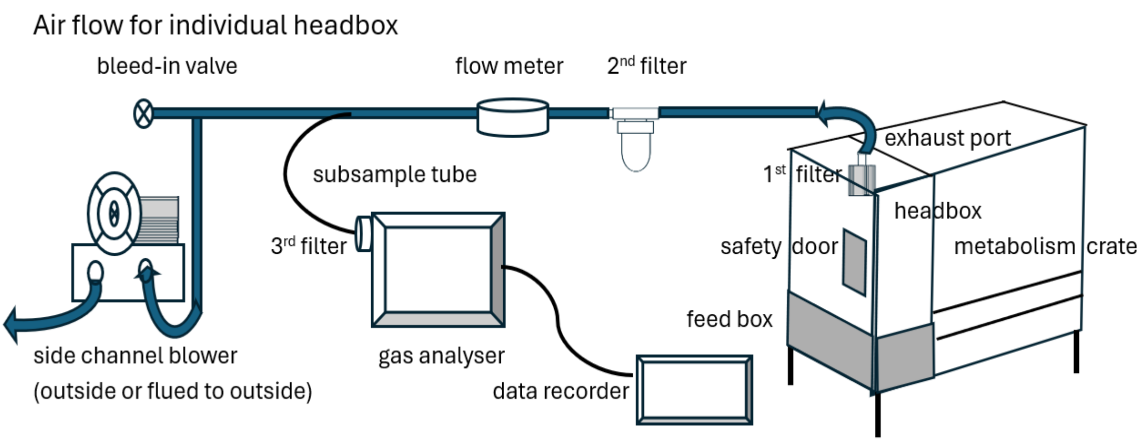
- Feed bins are not essential but are helpful for weighing, and feeding rations and weighing refusals
- Typically, the sides of the headbox around the feed bin are tin not plastic, but this is not critical.
- Subject to the type of feed, the volume of feed may be more than can be fitted in the feed trough 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 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:

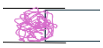
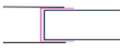
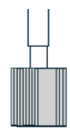
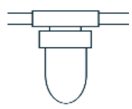
- The air is drawn out from the headbox via a manifold (PVC pipe 5 cm diameter for cattle) which fits to the exhaust port in the roof of the headbox. This can be either a 'T' across the top or an inverted 'U' coming down the sides as well. It is perforated with many holes to allow air entry and to ensure 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 5cm-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 the water in cow breath but this also reduces the ppm of CH_4 and CO_2 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 N_2, industrial N_2 is fine) & record there adding (hopefully 0 ppm CH_4).
  - Read a span gas sample (a standard analytical gas whose CH_4 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 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 [CO_2] 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 [CO_2]. 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^{o}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 measured wet-air airflow rate (e.g. 500L/min as measured to a dry-air flow rate 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 (2.1.1), 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 CO_2:

2.2.2.1 Release direct to intake pipe (CO_2):

- 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):

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 (2.2.2.1) or longer term (2.2.2.2) introductions of methane can be made as for CO_2. A rate of CH4 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.

2.2.3 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.

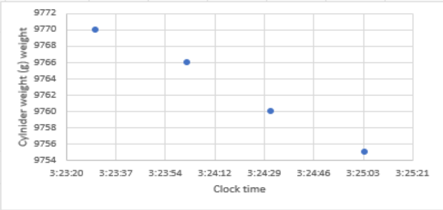
Cambodia Gravimetric Recovery test

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

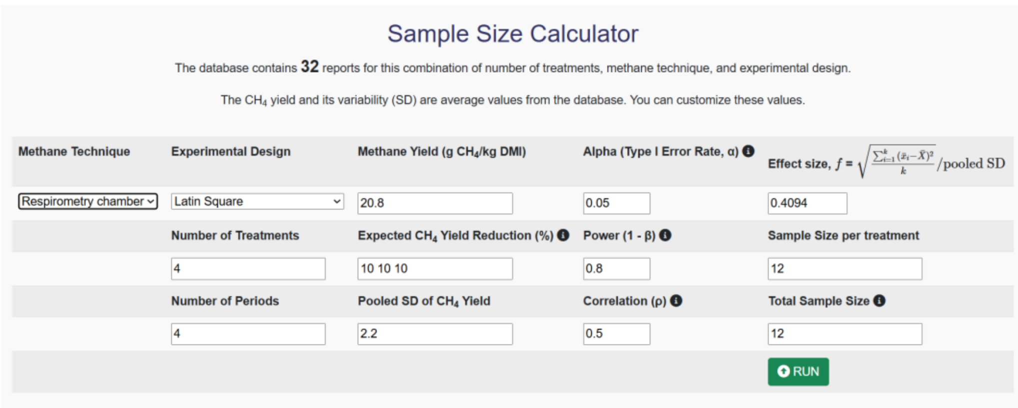
3. Animal Management

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/



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 CH_4 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 gCH_4/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 *adlib* 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 adlib 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 CH_4/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.

4.2 Corrections to data:

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

4.2.1 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:

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

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 CH_4 at STP to g CH_4.

4.2.2 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 CH_4 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:**

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

4.2.3 Correct for Gas Recovery, %

Gas recovery can be expected to be between 95 and 105% of that infused. Some laboratories accept that 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 recognize 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 cH4 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.

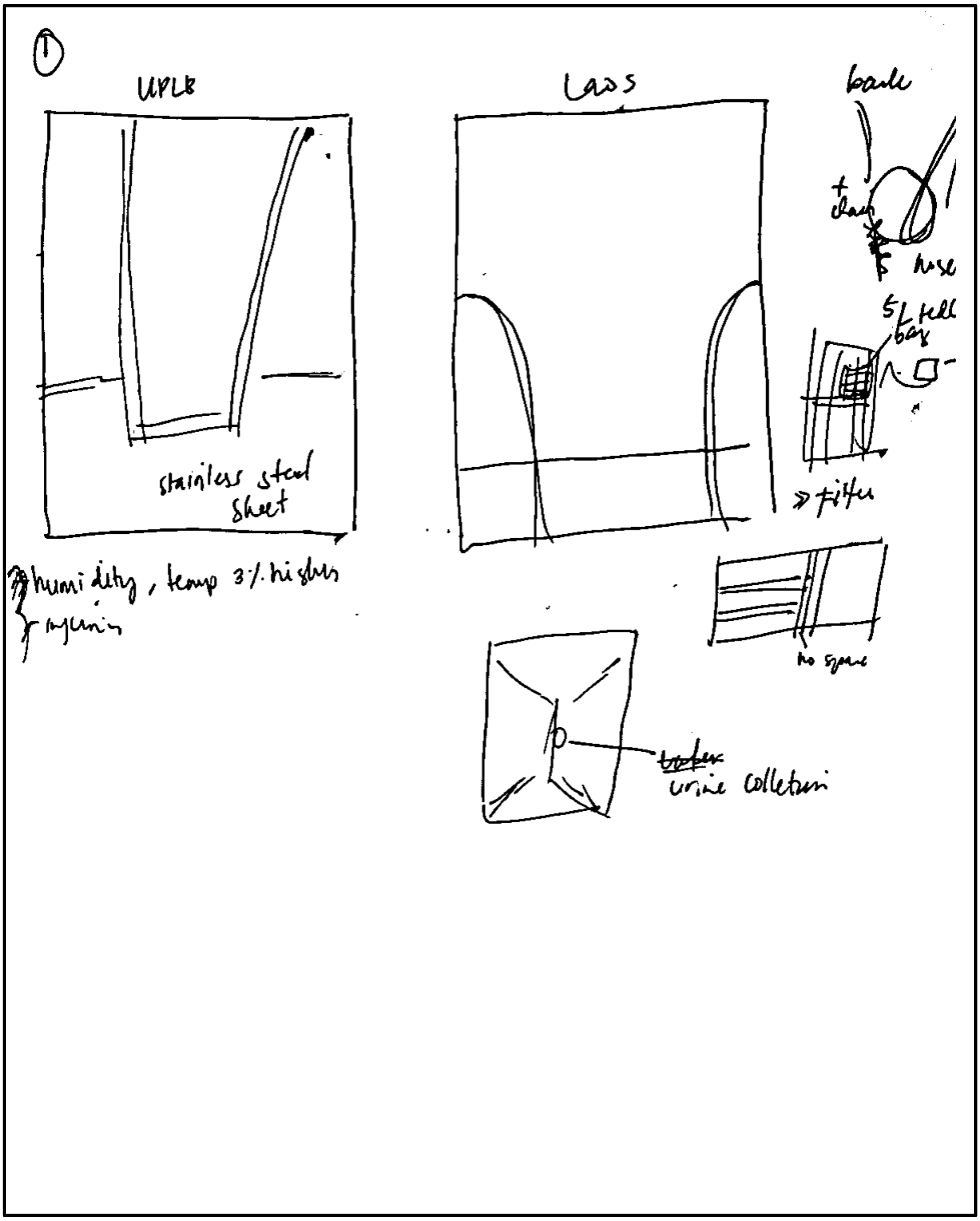
Appendix A. Headbox Challenges with Solutions

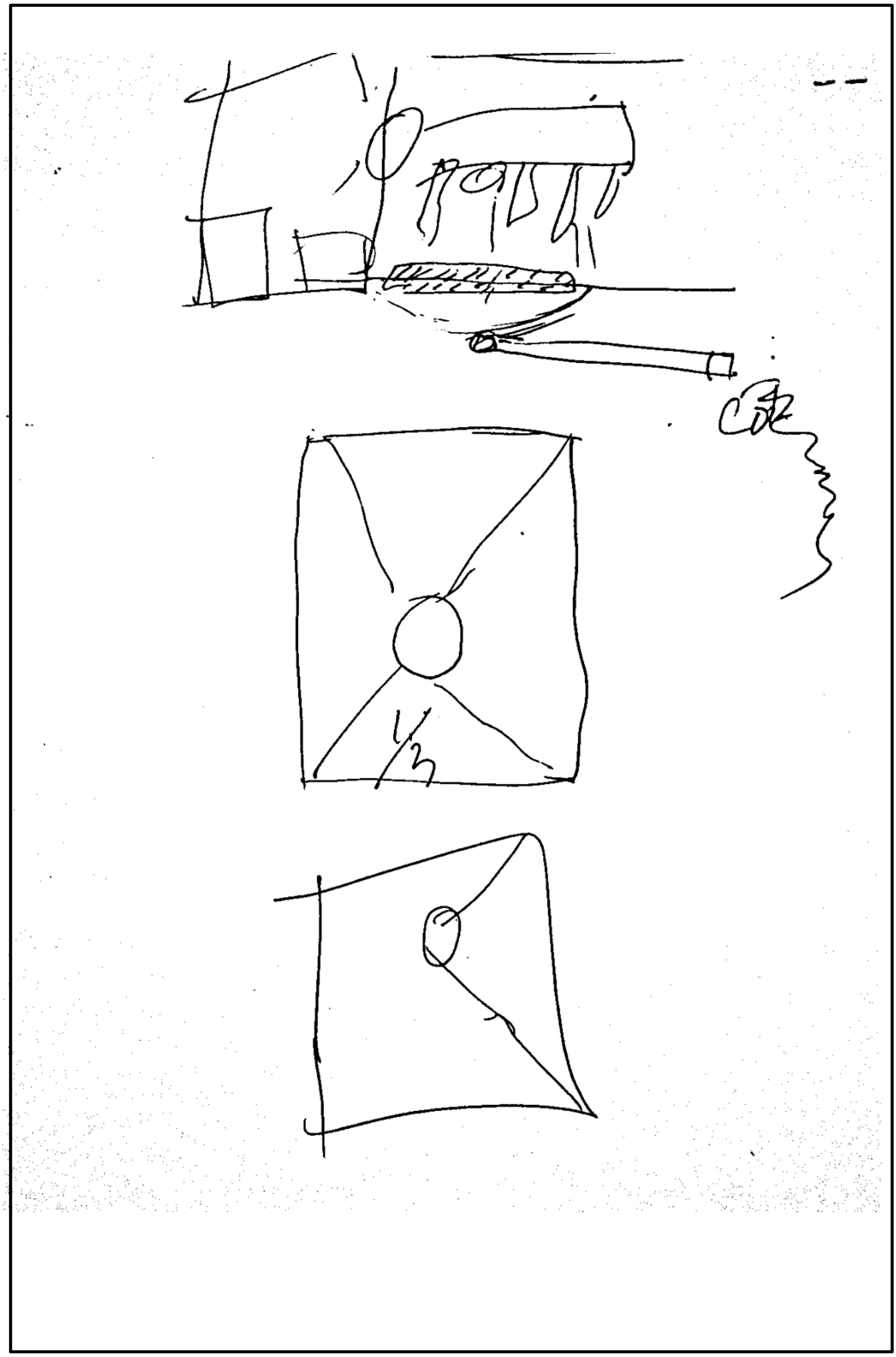
Experiences of users from the Headbox Workshop held in Phnom Penh, May 2025

ISSUE	CHALLENGE	SOLUTION
STRUCTURAL		
Old met crates, repurposed	Some injuries to animals	Smooth surfaces, use rubber matting & padding
Met crates high	Loading difficult	Lower met crates as much as possible; use ramps, pallets, bags, or trolleys
Concrete met crates	Not flexible but suitable for long-term housing; matting needed	
Metal met crates	Flexible but difficult for long-term housing, so training essential; matting needed	
Hood constrictive	Animals hot, isolated	Make 'funnel' wide and shallow, with clear material or inserts
OPERATIONAL		
High relative humidity	Condensation in HB & lines	Install dehumidifiers, increase air flow rate
High temperature	Animals off-feed & agitated	Install bigger fans, increase air flow
Dust entering ducting	Clogging, equipment issues	Install air filters, clean regularly
Leakage in ducting	Recovery rates low	Check regularly
Faeces in urine	Contamination for Total Collection	Regular/instant collection of faeces; matting, grates, good drainage
ANIMALS		
Cattle wild temperament	Hard to train, damage to HB	Persist with training, improve restraints (adjustable back rails, collar)
Cattle not trained	Restless in HB, go off feed	Lure into HB with fresh feed; have door open; gradually increase time in HB
Cattle restless in hood	Can't see other animals	Use some/all transparent material so animals can see each other
Animals vary in size	Hard to restrain	Use adjustable rails, tethers, chains
Cattle small	Can't lie down comfortably	Design back of head box with lower bar or cut it out
Animals with horns	Damage to HB	Dehorn well before trial, use younger animals,
Small herd available	Limited replication	Use Latin-square with covariate 'wash-out' period; block as much as possible
FEED & WATER		
Feed supply unreliable	Difficult to maintain quality	Contract with reliable farmers; use silage; store on site when possible

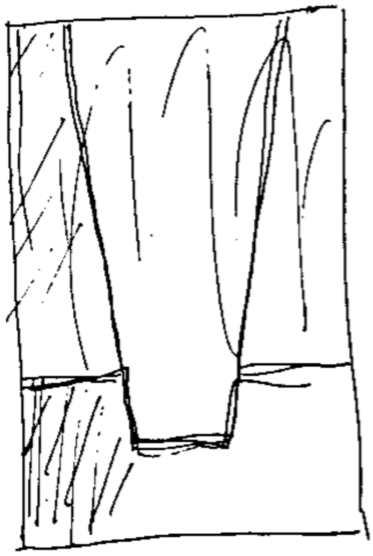
Bulky feed	Too much to feed once/day in HB	Feed twice/day, discard data from the period when doors are opened
Reaching all feed	Feed trays too deep, corners	Curved (1/2 drum) or circular feed trays; lengthen hood 'funnel'; loosen tether
No reticulated water	Need self-watering in headboxes	Install header tank for gravity fed water to individual troughs
Water slashed into feed	Feed wastage, DMI compromised	Install water lower than or far from feed tray, or block between them
Water splashed into HB	Increased humidity	Small drainage holes can be drilled where water pools
OTHER		
Electricity unreliable	Suffocation of animals	Safety doors installed; staff on-site always; alarm if power fails; generator
Security of equipment	Theft always possible	Lock gear in fixed metal cages or trolleys; students living on-site; lock gates

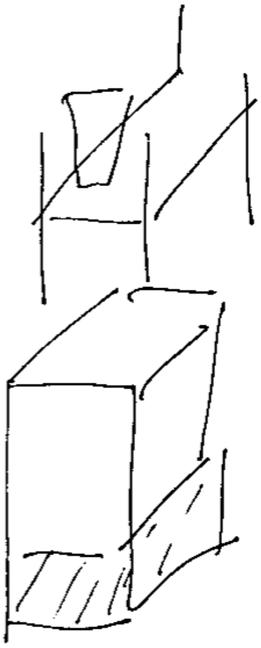
Appendix B. Headbox: Structure of Headbox Entrance with Hood Design + Metabolism Crate Design





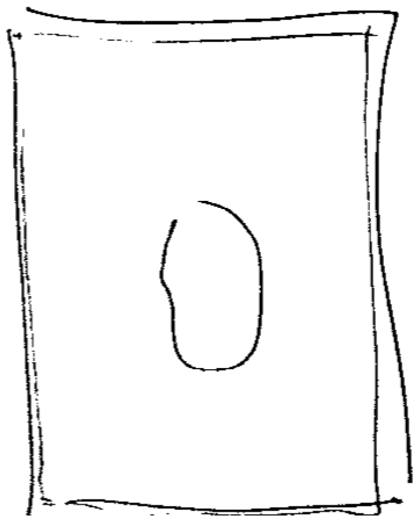
①

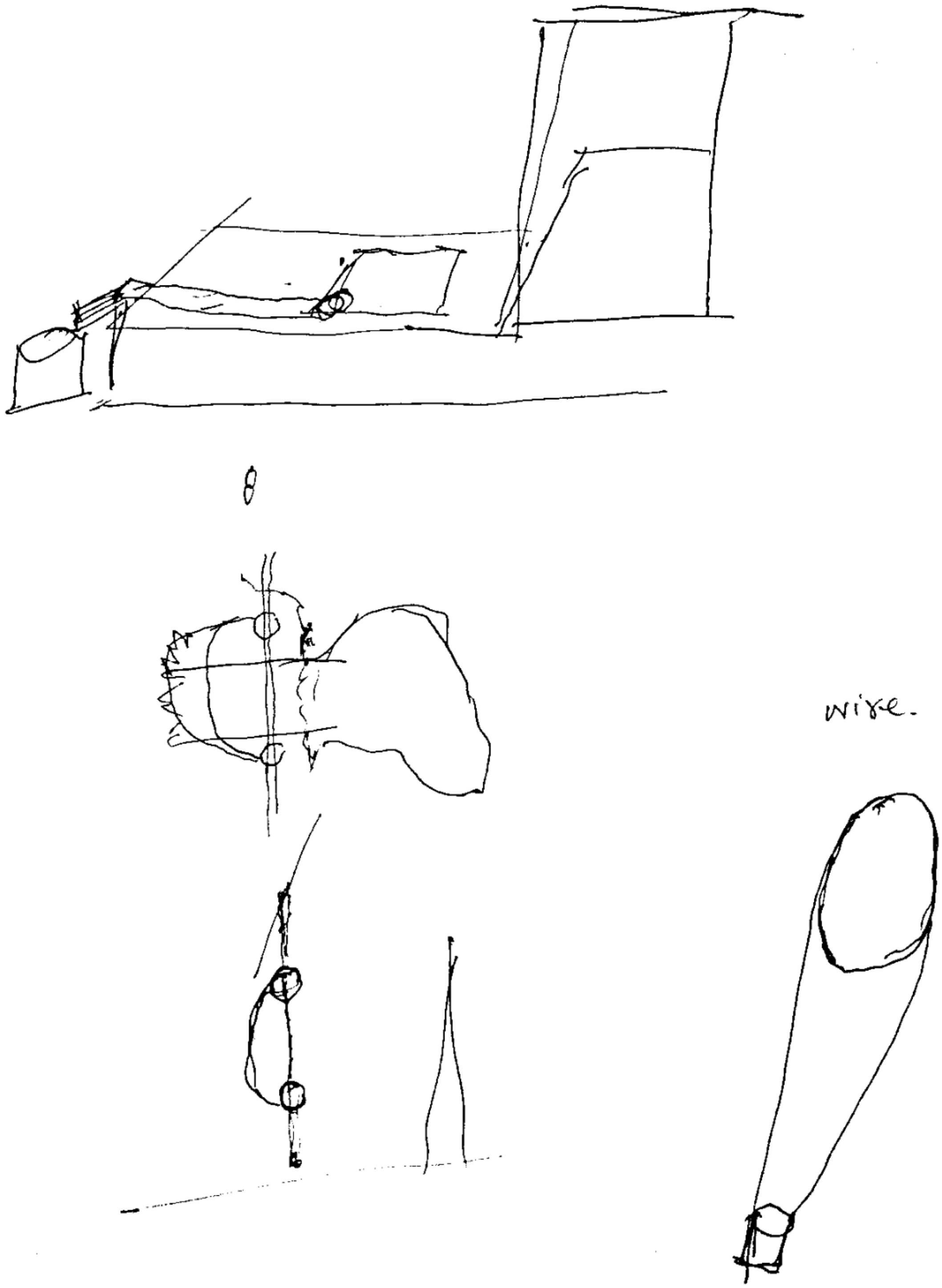


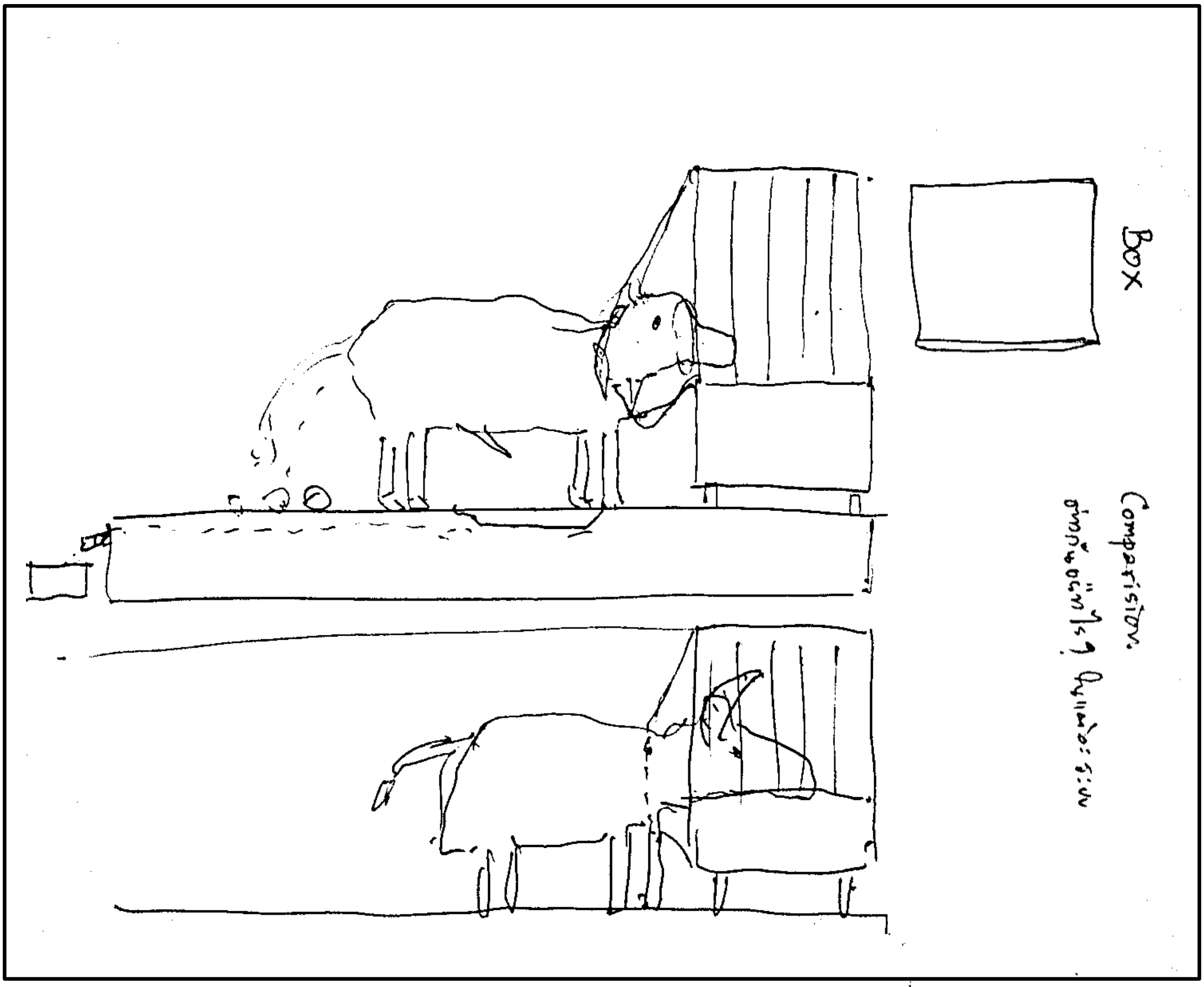


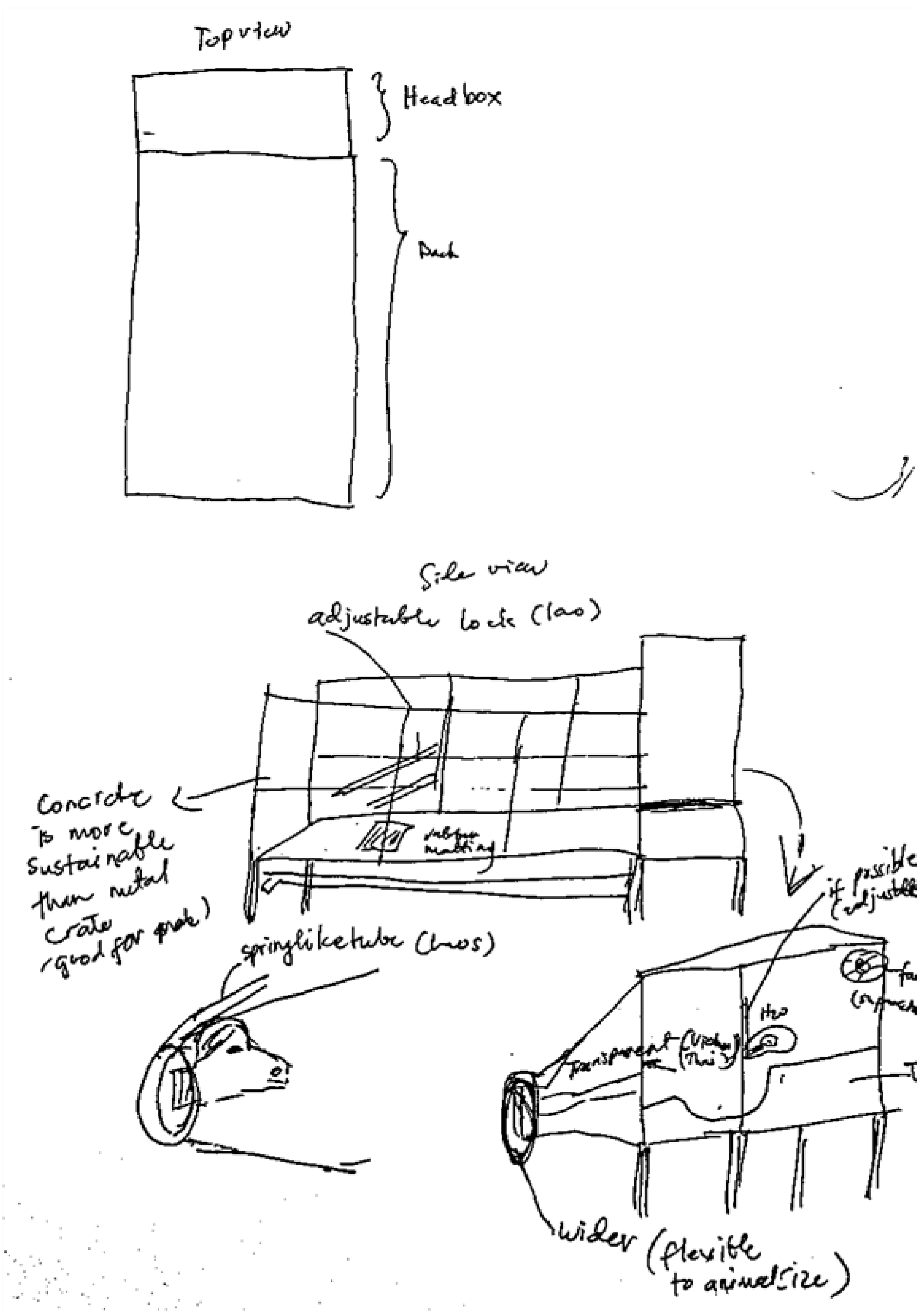
2 Illustrated challenges

CONSTANTINE.KATONGOLE@GMAIL.COM




wire.


Box
Comparision


Top view
Head box
Side view
adjustable lock (lao)
Concrete is more sustainable than metal crate (good for grade)
springliketube (laos)
if possible adjustable
transparent
wider (flexible to animal size)

Appendix C. Headbox Samples

Location: Indonesian Research Institute for Animal Production (IRIAP), Ministry of Agriculture, Indonesia

Scientist Responsible:	Slamet Widodo, M.Sc.; Dr. RA Yeni Widiawati
Email:	slam028@brin.go.id; raye001@brin.go.id

Description of facility:

IRIAP's headbox facility comprise

- Four (4) individual headboxes for large ruminant and four (4) individual headbox for small ruminant
- Large Animal Respirometry System for Ruminants from Sable System® which is consist of:

a. 5-2000 L Flow Generator (4 units)
b. Fixed Rate Pressure Pump (1 units)
c. Eight Channel Respirometer Multiplexer (1 units)
d. Gas Subsampler/Pump/ Mass Flow Meter (1 units)
e. Water Vapor Analyzer (1 units)
f. Carbon Dioxide Analyzer (1 units)
g. Methane Analyzer (1 units)
h. Oxygen Analyzer (1 units)
i. Computer for data acquisition and processing
j. Nitrogen UHP, pressure 150 Bar with purity >99.99%
k. Methane UHP with purity 99.95%
l. Methane
m. Carbon dioxide
n. Gas filter placed on the top of each individual headbox.

Principle of operation:

Summary: In a multiplexed large animal respirometry system using the Sable platform, each animal is placed in a sealed, ventilated chamber through which a known flow of fresh ambient air is continuously drawn. Air enters the chamber via a controlled inlet and exits through an exhaust outlet, where the outflow is routed through a mass flow controller to measure the exact airflow rate. A multiplexer unit sequentially selects one chamber at a time and directs the outflow air to high-precision gas analyzers that measure concentrations of oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4). Simultaneously, ambient air samples are also drawn in for baseline comparison. The gas concentration differences between inflow and outflow, combined with airflow measurements, allow for the calculation of respiratory gas exchange and methane emissions. The switching between chambers is fully automated and controlled by the system's software, with built-in flush cycles to prevent cross-contamination. All data, including gas concentrations, airflow, temperature, and humidity, are recorded and processed in real-time using Sable's ExpeData software to determine metabolic rates and emission profiles for each individual animal. System details are described below:

Headbox details: The headbox used measures approximately 200 x 120 x 300 cm (W x L x H) and is raised 30 cm off the ground to facilitate cleaning. It is constructed from welded stainless steel with varying thicknesses in different sections. In general, the headbox is divided into two main parts: the body and the head. The head section is equipped with a thick fabric partition that helps isolate air exhaled from respiration and eructation. The front and side panels are fitted with acrylic glass (approximately 1 cm thick), with the front panel designed to open for easy feed and water replacement. Above the head section, an air mixer and gas outlet are installed to collect exhaled gases, which are then directed to the gas analysis system.

Air flow: Ambient air is drawn into each sealed chamber through an inlet at 150L/minute, passes around the animal, and exits through an exhaust outlet. The outflow air moves through a mass flow controller and is pulled by a side-channel blower or vacuum pump. Airflow is continuous in all chambers. A multiplexer sequentially directs exhaust air from each chamber to the gas analyzers, while non-sampled chambers vent to the atmosphere. Flow rates are logged and corrected for temperature, pressure, and humidity.

Air Temperature and Pressure:
Air temperature and barometric pressure are continuously measured using dedicated sensors placed near the airflow system. These measurements are recorded alongside gas concentrations and flow rates. Data from the sensors are used for real-time correction of gas volumes and flow, ensuring standardized conditions across all chambers.

Gas Analysis:

a. **Air Routing**: Exhaust air from each chamber is routed through the multiplexer, which selects one chamber at a time to direct its air to the gas analyzers.
b. **Gas Measurement**: The gas analyzers continuously measure the concentrations of gases: **Oxygen (O_2)**, **Carbon Dioxide (CO_2)**, and **Methane (CH_4)** in the exhaust air.
c. **Concentration Difference**: The system measures the difference in gas concentrations between the incoming fresh air (inflow) and the outgoing exhaust air (outflow). This difference reflects the gas exchange happening within the chamber.
d. **Calculation of Gas Exchange Rates**: The gas exchange rates are calculated based on the concentration differences:
    - **O_2 consumption (VO_2)** is determined by the depletion of oxygen.
    - **CO_2 production (VCO_2)** is determined by the increase in carbon dioxide.
    - **Methane emission (VCH_4)** is measured directly.
e. **Real-Time Data Logging**: Gas concentration data from the analyzers are continuously logged and stored by the ExpeData software, which time-stamps and synchronizes the readings for each chamber.
f. **Temperature, Pressure, and Humidity Corrections**: The gas measurements are corrected for environmental conditions, including **temperature**, **barometric pressure**, and **humidity**, using dedicated sensors. These corrections ensure that the gas volumes are measured under standardized conditions for accuracy.

Data Processing using ExpeData: ExpeData software continuously acquires real-time data from gas analyzers, flow meters, and environmental sensors, such as temperature, pressure, and humidity. The software synchronizes these data streams, ensuring that readings from different sources are time-stamped and aligned for each chamber during its turn in the

multiplexing cycle. Calibration factors are applied to correct the raw data, adjusting for any drift in gas analyzers or flow meters. The software then calculates gas exchange rates, including oxygen consumption (O_2), carbon dioxide production (CO_2), and methane emission (CH_4), based on the differences in gas concentrations between inflow and outflow air, corrected for airflow. Real-time monitoring is facilitated by ExpeData's graphical display, showing dynamic changes in gas exchange rates and environmental conditions. Once the data is processed, ExpeData allows for the export of results in formats such as CSV or Excel for further statistical analysis and reporting. The software also includes diagnostic tools to monitor the system's performance, ensuring data quality and system integrity.
Gas recovery procedure: The gas recovery procedure is performed by releasing high-purity methane (CH_4; purity >99.95%) into each chamber for a defined duration. Recovery efficiency is quantified by calculating the difference between the total volume of methane introduced and the volume recovered, with concentration measurements used to verify gas capture accuracy.
Photo library of your system:
Photo of headbox  **Front view of headbox chamber**

Feed bunk and water container

Rear view

Photo of airflow components

- Blower fan and any air bleed-in valves

Blower and fan for air inlet

- Flow meter

Photo of gas analyser & and filter/dryer components

Control room

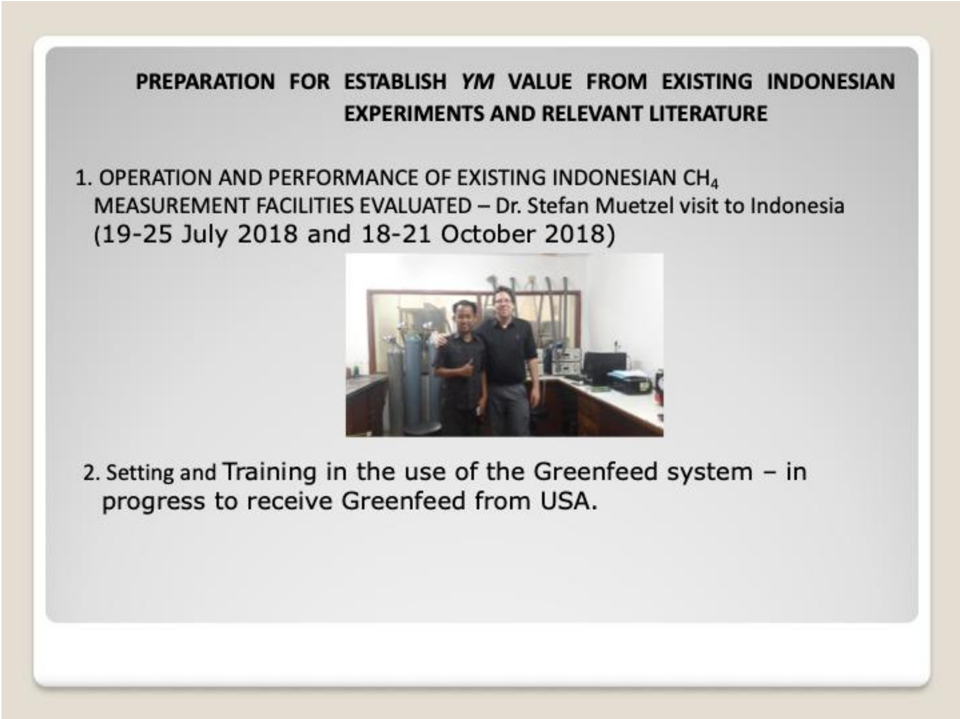
Additional Photos:

A. Sable system installation at IRIAP


MA-10
CA-10
PA-10
MUX
FLOWKIT
FLOWKIT
FLOWKIT
FLOWKIT

Dr. Stefan Muetzel (AgResearch NZ) as a trainer. Participants were scientists from research institutes and universities

Training in how the head box system work and calculation then analyzed the results



For comparison, the experiment was conducted by using a head box chamber connected to a methane analyzer, with the same diets offered as performed in the Greenfeed and Smartfeed Pro systems.

B. Methane measurement using a portable headbox chamber during field research on beef cattle


Farmer group (Yogyakarta Province)

Location: Royal University of Agriculture, Phnom Penh, Cambodia

Scientist Responsible:	Assoc Prof. Sath Keo
Email:	keosth@gmail.com

Description of facility:

RUA's headbox facility comprise

- Feed bunk adequate to feed and record intake of 12 cattle individually.
- Four (4) headboxes with associated metabolic crates for collection of faeces and manure.
- The air flow and gas sampling system are contained in a mobile cart so these valuable components can be protected from theft when no experiment is in progress.

Principle of operation:

Summary: The four headboxes all operate independently. Air is continuously drawn from the top of each headbox into an exhaust pipe then through an in-line recording air flow meter and into a side-channel blower that draws the air then voids it into the atmosphere. As air is drawn out the exhaust pipe from the headbox, fresh ambient air is drawn into the headbox around the loose-fitting shroud. A continuous subsampling of exhaust and ambient gas into Tedlar bags is made using miniature peristaltic pumps. Each bag of subsampled gas (1 per headbox plus one ambient air bag) is subsequently analysed by a multi-gas analyser. System details re as below:

Headbox details: 90 x 80 x 110 cm box, raised 30 cm off the ground. Box is made of welded steel covered with 6mm thick polycarbonate. Door is secured by 3 'butchers latches' to make airtight seal and has foam strip between door and box to ensure airtight seal. The shroud dimensions are as shown in Photo 1.

Air flow: Air is drawn through each headbox by a Side channel blower (Ventex 2RB010-7AA11; purchased from "Topgas" in Thailand), at a rate of 500L/minute. This is the maximum flow possible with this pump Flow rate is varied by a 'T-piece' fitted I the pipe between the flow meter and the blower, with a gate valve on the stem of the 'T' that is open to the atmosphere. By opening this gate vale, ambient air is allowed into the blower, reducing the amount of air being drawn from the headbox & through the flow meter. The flow meter is an Aichi Tokei TBX-30L that has a self-contained battery with 7-year life. The display on the in-line meter is recorded at the start and end of the 23h measurement period and divided by time elapsed to calculate flow rate.

Air Temperature and Pressure:

Because the Aichi Tokei TBX-30L turbine flow meter is NOT temperature and pressure corrected, we need to measure the temperature and pressure of air going through the flow meter. This is done by inserting a hand-held temperature and pressure gauge into a sampling port in front of the flow meter. This temperature and pressure is read 3 times per day and the values written down in the experiment log sheets. Typically, pressure is only 3-6kPa less than the atmospheric pressure (approx. 100kPa depending on altitude))

Air Sampling: A continuous sample of air over 23h from when the chamber doors I closed is collected into a pre-evacuated 5L tedlar bag. The sample bag is left sealed and connected via tygon tubing to a miniature peristaltic pump (LONGER Peristaltic Pump Miniature BQ50-1J adjusted to pump at 2-3 ml air/minute. The bag is opened and the pump turned on when the headbox door is closed. The bag is pulled closed to seal 23h later and taken for immediate analysis.

Gas analysis.

The gas is analyser using an Aquagas GA40T+ multigas analyser, that reports O2, CO2, and CH4 gas levels directly on-screen. It is not linked to a computer. The analyser draws approximately 1.5L sample/minute and is used as below:

- The analysis is done in a well-ventilated room well away from cattle or other sources of methane (this is so the background CH4 level is truly 0ppm)
- The analyser is allowed to warm up for AT LEAST one hour
- The **'zero standard'** (pure N2) and the **calibration/span gas** (CH4 in N2) are run through the analyser. Ideally they should be transferred into evacuated tedlar gas bags directly from the cylinder and then let the gas analyser internal pump suck them from tedlar bag into the gas analyser. Write down the expected O2, CO2 and CH4 concentration in each gas and the observed concentrations.
- Then connect each of your 5 bags gas (background bag + 4 headbox bags) and le the sample be draw in for 40 seconds – the reading should have stabilised on the analyser display by then. Write down or print the results foe all 3 gases)
- Then repeat the measurement of each bag, recording the concentrations shown 40s after connecting each bag.
- The repeat the zero standard and the calibration gas bags.
- LOOK at the calibration bag results ~ was it within 10% of the value shown ion the cylinder itself? If it is crazy different we have a problem. If it is <10% different then we can correct ALL the gas bag readings by whatever the % difference is. So if the calibration gas was meant to be 1000ppm but we read it as 950ppm, the value is 5% low, so we would multiply up all the other CH4 ppm values by 5% before we use them.

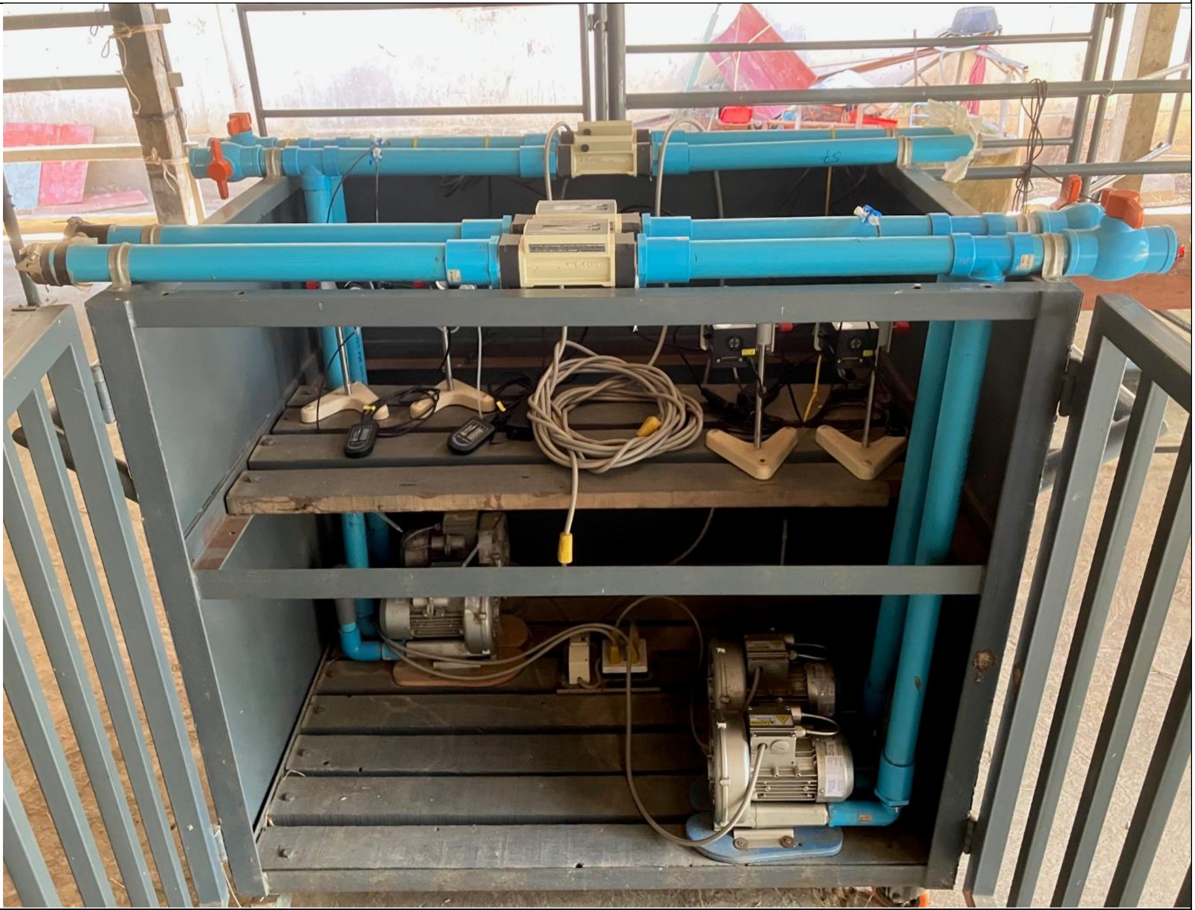
Gas recovery procedure:

A short term 3-min recovery of tracer gas (CO2) delivered up the exhaust outlet is used, with the rate of weight loss of the (small) cylinder being multiplied by the flow rate to work out the expected (100% recovery) increase in CO2 level expected and this s compared with the ppm of CO2 observed. Co2 is released from a small cylinder at a fixed rate of 10-15g/min using a regulator with a rotameter. The cylinder is placed on an electric scale reporting to 1g and every 30s the cylinder weight and the CO2 in the exhaust air is recorded.

Photo library of your system:

A summary of photos of key components of the system is provided below

Ball valve that allows the balance of air drawn from the he4abdox and from the outside atmosphere to be varied to ensure flow through the headbox is approximately 500L/min



The core of the headbox system is the mobile airflow and analysis hub. This system can be closed off for security but in it is housed
4 side-channel blowers (on floor of box) connected to the 4 air flow ducts (blue pipe) running along the top), which have a “t’ piece junction and a ball-type valve to allow outside air into the blowers and thus reduce the amount of air drawn from the headboxes. Fitted into the air flow system across the top of the trolley are 4 turbine flow meters. Narrow diameter tube samples air from each the 4 blue pipes and this air is pumped by a 4 mini peristaltic pumps (seen with V shaped stands on middle level) and into a tedlar gas collection bag (not shown)

Site of sampling ports in the air flow pipelines. The sampling tubing is connected to the peristaltic pump tube with peristaltic pump collecting 2-2.5 mol/min of air continuously.

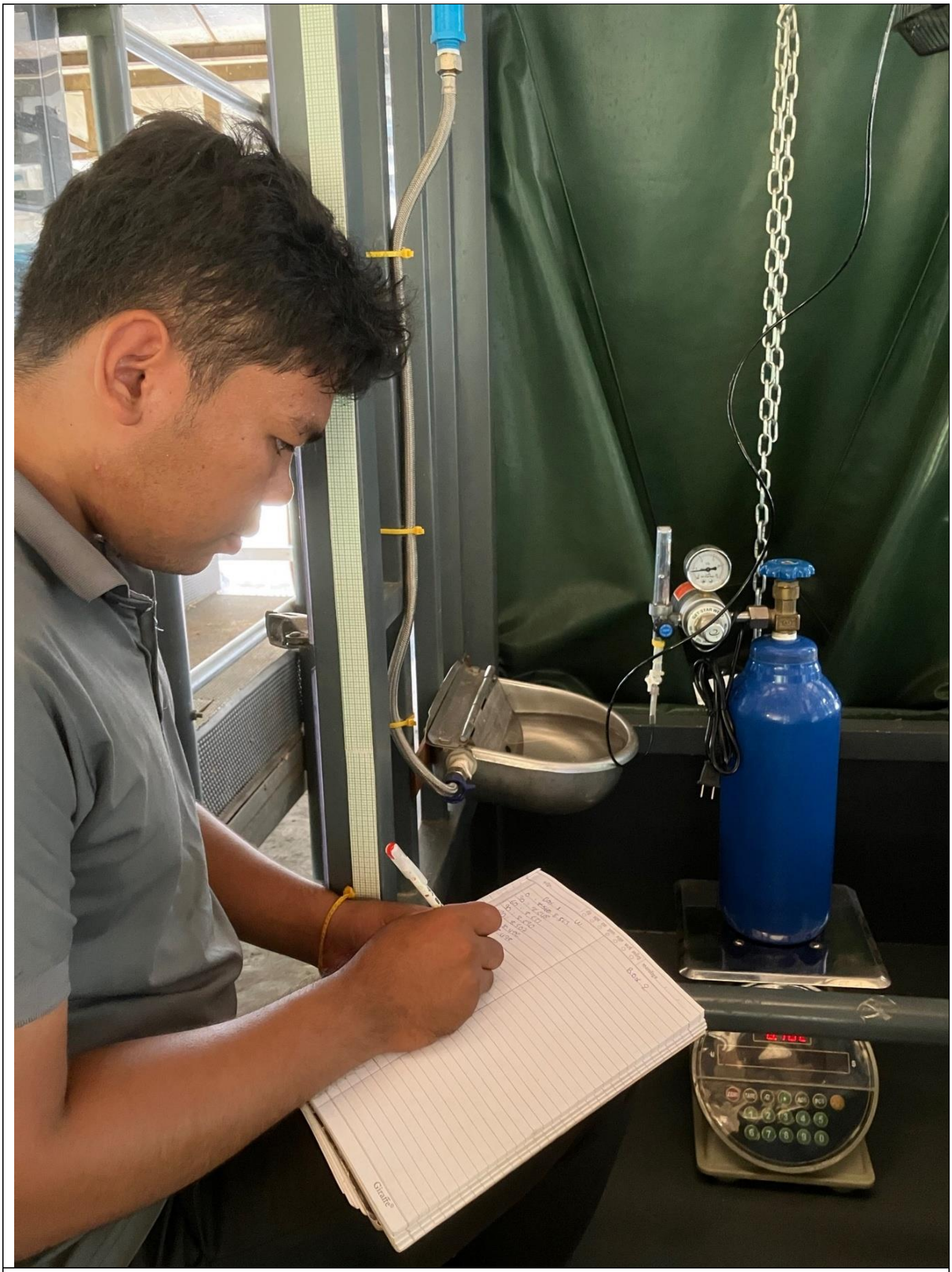
Shroud through which the cow places its head to enter the headbox

View of the inside of the headbox from the front door. Note bar across front which is used to tether cattle to and chain hanging down that can be used to secure the animal from excessive movement.

Metabolism crates fitted to headboxes to allow collection of manure and urine for nutrient digestibility and excretion sample collection

Battery operted turbine flow meters. These can be re-zeroed before each day's study

Front view of the chamber showing the blue flexible ducting used to connect the exhaust port (top centre of headbox) to the analysis trolley (shown disconnected at right)



Student reproting the weight of the small CO2 clylinder releasing 5L/min over 3 minutes while othe sreord the average [CO2] in the airstream leaving the headbox

Location: University of the Philippines Los Baños, Laguna, Philippines

Scientist Responsible:	Gerard F. Guadayo
Email:	gerardguadayo@gmail.com

Description of facility:

UPLB's headbox facility comprise

- Features elevated metabolism stalls fitted with fully enclosed headboxes made of polyacrylic and stainless-steel materials.
- Headboxes isolate the animal's head and are sealed using adjustable vinyl tarpaulin neck coverings/hood
- A pipe network equipped with blowers and flow meters maintains consistent airflow and directs captured gases to the gas analyzer (SmartCEMS Multiplexer Gas Analyzer)

Principle of operation:

Summary:

The UPLB headbox respiration facility includes four sealed headboxes operating independently to measure GHG emissions from cattle. During measurements, air is continuously drawn from the enclosed head area of each animal through two vertical intake pipes positioned near the breathing zone that merge into a main pipe connected to a blower system. As air is drawn out, ambient air enters the headbox through the adjustable neck shroud. A portion of the airflow is continuously sampled via small tubes inserted after the airflow meters, which lead to a multi-channel gas analyzer. In this system, four headboxes and one ambient air channel are monitored sequentially. Negative pressure is maintained inside each headbox to prevent leakage and ensure accurate sampling of exhaled gases

Headbox details: The metabolism stalls are elevated approximately 50cm above the ground and measure 365cm in length, 95cm in width, and 145cm in height (without the box). Each headbox is detachable from the stalls and measure 90cm in length, 105cm in width, and 160cm in height. They are constructed using stainless steel frames combined with polyacrylic panels to create a rigid and sealed environment around the animal's head area. The animal's neck passes through a black vinyl tarpaulin shroud fitted with adjustable drawcords, allowing a loose but controlled entry of ambient air while maintaining negative pressure inside the box. Each stall is designed with a slatted floor with rubber padding to allow waste to pass through and be collected below. Feed bins and automatic waterers are provided inside each headbox to ensure that animals have access to feed and water during confinement. To restrict movement and prevent the animals from leaving the headbox, a neck collar system is used, with the collars secured externally to prevent pushing.

Air flow: Air is continuously drawn from each headbox by side-channel blowers operating at an approximate flow rate of 500L per minute per headbox. The intake begins at two one-inch vertical pipes on the sides of the box with multiple perforations, positioned close to where the animal exhales, merging into two-inch diameter main pipes. Flow rates through each channel are monitored by ATZTA TBX-D digital flow meters and adjusted manually via flow valves. Prior to experiments, airflow rates across all headboxes are equalized as closely as possible to minimize system variability. Temporary sampling ports located along the pipes allow the measurement of air temperature and pressure when necessary for corrections.

Air Temperature and Pressure: The air flow meters and gas analyzer readings are affected by temperature and pressure conditions so measurements must be corrected to STP to ensure accurate calculations of gas emissions. During the experiments, the sampling ports after the airflow meters are used for manual insertion of a temperature and pressure probe. These values are recorded and used to adjust the measured airflow rates and gas concentrations using Boyle's Law. Corrections ensure that all gas volumes and concentrations are reported under STP conditions, minimizing variation caused by environmental fluctuations inside the pipes.

Gas analysis.

Gas concentrations are measured using a SmartCEMS Multi-gas Analyzer manufactured by AquaGas. This analyzer is equipped with an automatic multiplexer capable of sequentially measuring gases from four animal channels and one ambient air channel. The system operates in flip-flop mode, with each cycle lasting 15minutes, comprising 5 minutes of ventilation (zeroing phase), 2.5 minutes of infusion (purging phase), and 7.5 minutes of active measurement. The analyzer measures methane in ppm, carbon dioxide in percentage, and oxygen concentration. The analyzer is placed inside a separate, ventilated analysis room to prevent contamination from external methane sources.

Gas recovery procedure:

Before conducting experiments, a gas recovery test is performed to assess the integrity of the system. Pure methane gas (99.99% CH_4) is released from a high-pressure gas cylinder through a two-stage regulator and mass flow controller, set to deliver gas at 30 Ncc/min under normalized conditions. The methane gas is injected directly into the headbox system via one of the pipe's perforations. The mass flow controller is configured to reduce the inlet pressure to a safe operational pressure of around 30 psi. After a brief stabilization period, readings from the gas analyzer are taken and adjusted for ambient temperature and pressure using Boyle's Law. Ambient methane levels, measured before the infusion of the known gas, are subtracted from the experimental readings. The recovery percentage is then calculated by comparing the measured concentration to the theoretical concentration derived from gas flow rates. A recovery range between 95% and 105% is considered acceptable. If the recovery rate falls outside this range, the system is inspected for leaks using smoke tests, visual inspection, or soapy water application. Identified leaks are sealed using appropriate sealants, and in cases of significant damage, replacement of affected piping sections is performed.

Photo library of your system:

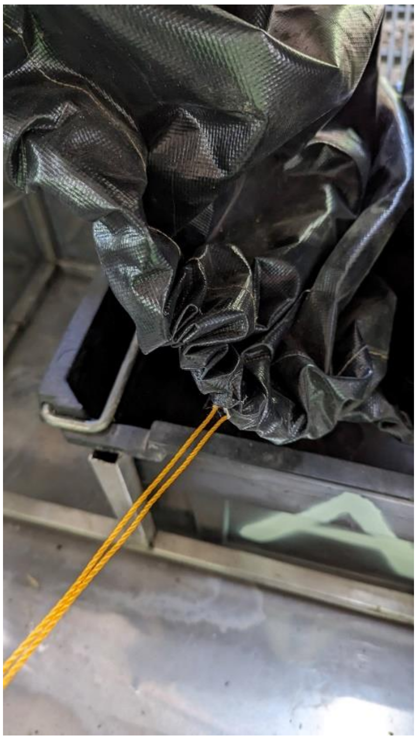
Photo of headbox:

a. Exterior and interior of the headboxes

The four metabolism stalls equipped with headboxes at the facility

c. Rear of the headbox, slatted flooring of the stalls, and the ramp used for transferring animals

d. Automatic waterer and feed bin installed inside the headbox

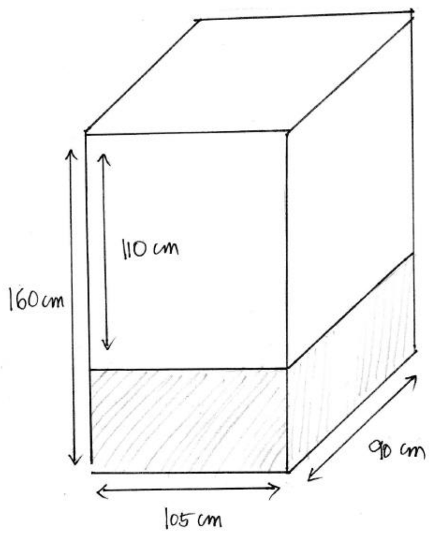


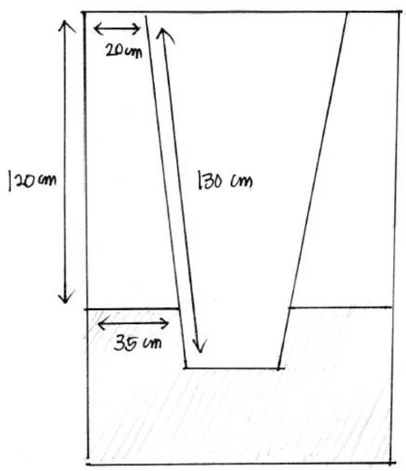
e. Shroud made of vinyl tarpaulin, and its drawcord

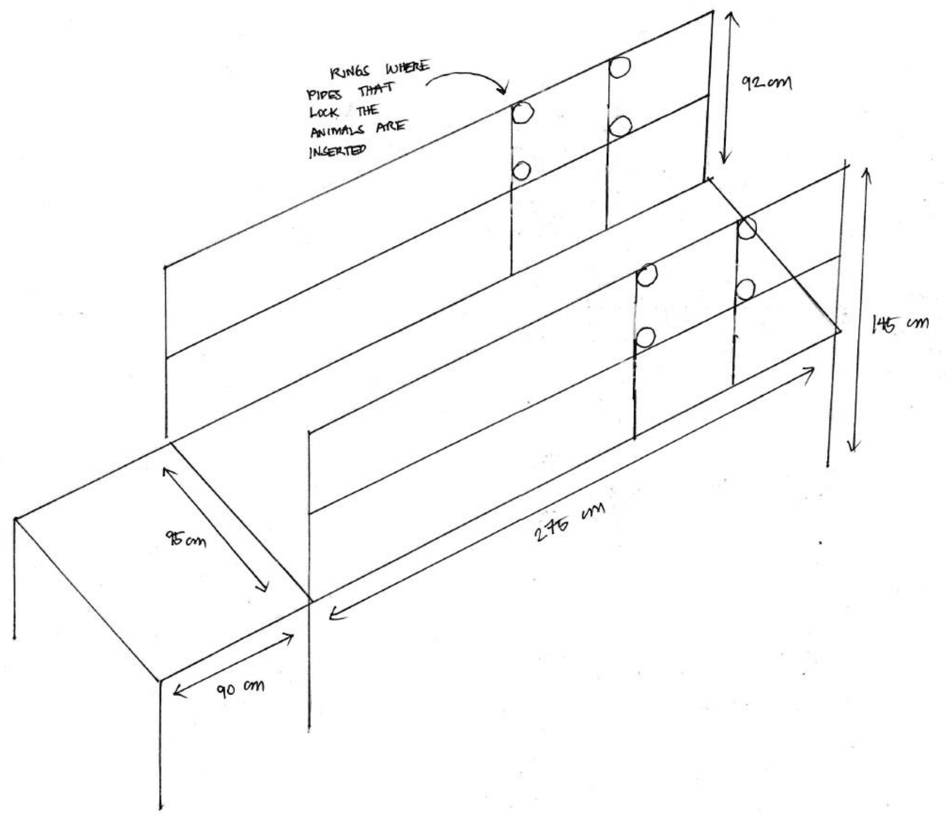
f. Vertical 1-inch pipes with holes for the entry of air to be analyzed

g. Main pipes (2-inch diameter) which direct the air towards the analysis room

Sketch with measurements of headbox:







Manufacturer's template for neck shroud construction:

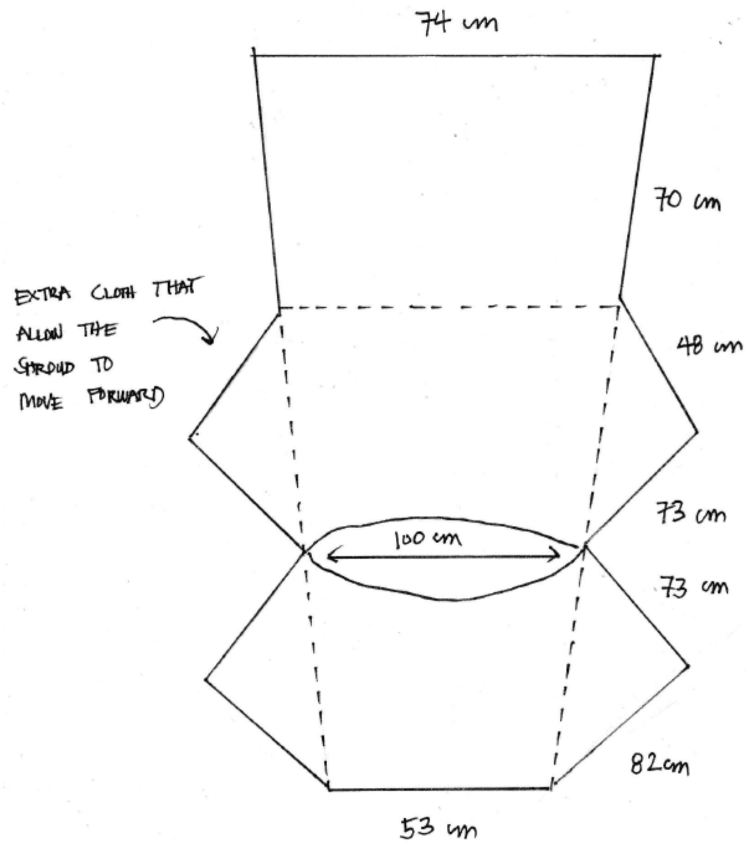


Photo of airflow components:

- Blower fan and any air bleed-in valves:

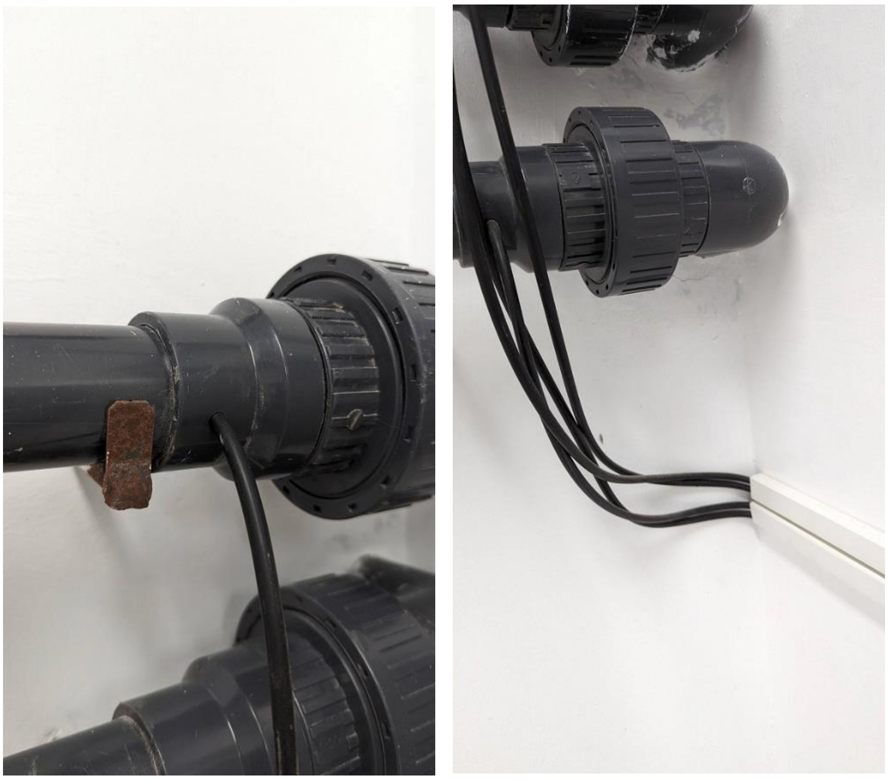
The blowers that draw air from the headbox into the gas analyzer, and out towards the environment

- Flow meter:

Airflow meters responsible for measuring the airflow in the pipes

Photo of gas analyser & and filter/dryer components:

j. Air filters

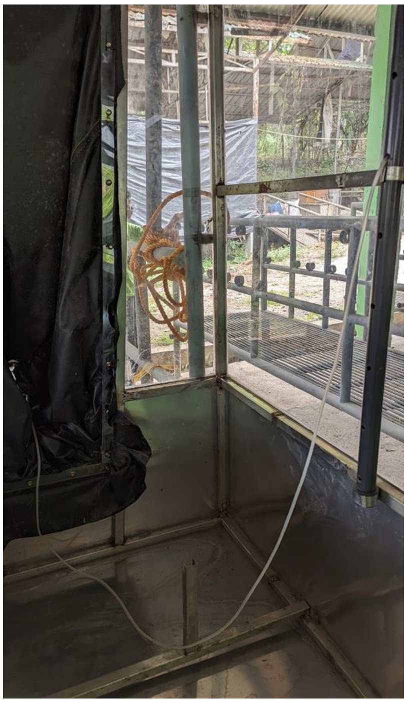


k. Sampling points and sampling tubes that lead to the gas analyzer

The gas analyzer with multiplexer used at DTRI - UPLB

Photo of gas recovery in action.

m. The mass flow controller used to infuse a known and steady amount of gas into the system



n. Set-up of recovery test wherein the gas is directly infused into one of the inlet pipes.

END

Location: Ruminants Feeding Standard Research and Development Center, Khon Kaen, Thailand & Bureau of Animal Nutrition Development, Department of Livestock Development, DLD, Thailand

Scientist Responsible:	Wanna Angthong, Thanamon Buranapawang
Email:	nungs11@gmail.com, dld.bui@gmail.com

Description of facility:

DLD's headbox facility comprise

- Feed bunk adequate to feed and record intake of 16 cattle individually.
- Four (4) chambers with associated metabolic crates for collection of faeces and urine.
- The air flow system, gas analyzers and computer are in the control room.

Principle of operation:

Summary: The principle of operation for Ventilated hood-type respiration calorimeters is based on an open circuit system and real-time data recording. Gas emission was measured by the head-hood system installed for 3 days each cow each period. A constant airflow from the animal chamber into the measurement system was controlled by blowers (Air suction pump) installed at the end of the system which the flow meter used to measure the air flow rate through the main tube. Then a small pump draws a sample of this air and directs it into each gas analyzers: Oxygen analyzer, Carbon dioxide analyzer and Methane analyzer. The values measured by the air flow meter and the gas analyzers are analog electrical signals then sent to a receiver and signal converter (A/D converter), which transforms them into digital values. Finally, these digital values are transmitted to a computer and stored using specialized software. (Open-circuit system with ventilated hood-type respiration calorimeters with details of the system structure according to the report of Suzuki et al., 2007, Suzuki et al., 2008). (Fig.1) System details re as below:

Headbox details: 80 x 105 x 173 cm box, raised 7.5 cm off the metabolic crates that raised 33 cm off the ground. (Fig.2, 3) The front of the cage has a square box made of stainless steel and has a clear acrylic sheet. It is a component that allows the view inside the box to help reduce stress for the animals. The animals are held in place by a cloth around their necks to hold the front of the animals inside the enclosure, which is similar to a hood. (Fig.4) Inside the enclosure are feed bunk and an automatic water bowl. (Fig.5) The back of the cage is an open area where the animals can stand or lie down normally.

Air flow: Ambient air flows through the hood into the chamber in one direction only, with no backflow that is controlled by a blower (Air suction pump) installed at the end of the system. (Fig.6). The vortex blower dawns air from each animal chamber, through the upper air pipe into the main tube, then blow out through the other port of the blower. There's the flow meter used to measure the air flow rate through the main tube that can range from 100 to 700 liters per minute. (Fig.7) The display on the flow rate meter is recorded at the start and end of the 23h 30m measurement period and divided by time elapsed to calculate flow rate.

Air Temperature and Pressure: The air flow meter is a thermal flow cell type with

temperature compensation and is suitable for measuring very low flow rates such as cow breath to minimize these effects and ensure accurate readings under varying. Air Sampling: A continuous air sampling for 23h 30m after closing the door. Once the air enters the main tube, a small pump draws the air sample through the auto gas sampling unit (Fig.8a, 8b), sends to the gas cooling dryer (Fig.9), then through the needle valve flow meters (Fig.10) into each gas analyzers including the Oxygen analyzer, Carbon dioxide analyzer and Methane analyzer. (Fig.11) Each gas analyzer has its own dedicated pump. Gas analysis: The concentrations of O_2, CO_2 and CH_4 from respiration were measured continuously by each gas analyzer. Oxygen analyzer is paramagnetic system but carbon dioxide and methane analyzer are a non-dispersive infrared sensor (VA-5000 series, Horiba, Japan; IR-200, Yokogawa Electric, Tokyo). The values measured are analog electrical signals then sent to a receiver and signal converter (A/D converter), which transforms them into digital values. Finally, these digital values are transmitted to a computer and stored using specialized software. This system records data in real time, continuously recording over 23 hours per day for 3 days per cow. Therefore, before starting the operation, the gas analyzer must be calibrated every morning before closing the chamber. There are the following methods: ▪ Warm up the entire system for at least 1 hour. Turn on the computer to open the dedicated software (GASMET, developed by Dr.Tomoyuki Suzuki) for recording data, such as the volume of oxygen gas, carbon dioxide gas, methane gas, and the air flow rate. (Do it only on the first day of each period) ▪ Before closing the chamber every morning, calibrate the three (3) gas analyzers: Oxygen analyzer, Carbon dioxide analyzer, and Methane analyzer. Use standard gases specific to each gas type at the highest level (Span gas) and the lowest level (Zero gas). These values must be accurate according to the standard certificate attached to each gas cylinder.
Gas recovery procedure:
Before the operation, the whole system check must be performed with pure carbon dioxide gas (CO_2, 99.99% purity) to check for gas leakage in the whole system. The recovery check value must be in the range of 95-105 % (Suzuki et al., 2008). After power on the GASMET program in computer and already calibrate the gas analyzers. Then, close the test chamber door, activate the blower adjusting the airflow for the anticipated animal size. Introduce carbon dioxide into the sealed chamber at a rate of 200 grams over 20 minutes, recording the initial cylinder weight. After 20 minutes, stop the gas flow, weigh the cylinder again to determine the released amount, and continue recording carbon dioxide levels until they return to the external air concentration. The volume of carbon dioxide measured by the carbon dioxide analyzer is in units of liters. It must be adjusted to grams then calculated as follows: Recovery (%) = Measured Carbon Dioxide Weight (grams) x 100 / Carbon Dioxide Weight Released from Cylinder (grams)
Photo library of your system:

Photo of headbox

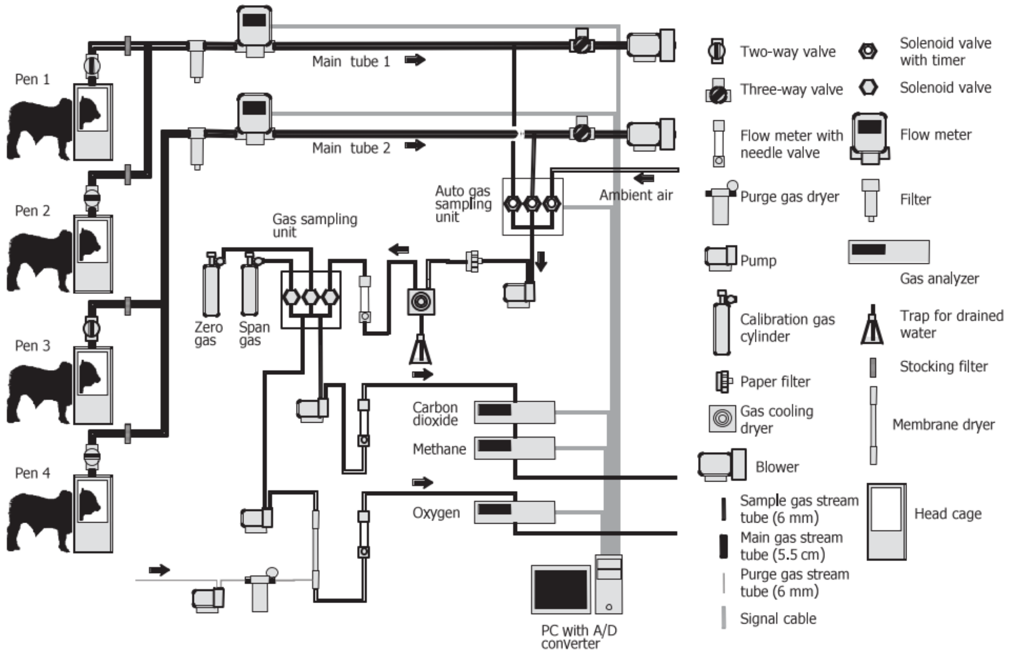


Fig. 1. Schematic diagram of the ventilated hood-type respiration chamber system
The black arrows indicate the direction of airflow through the system.

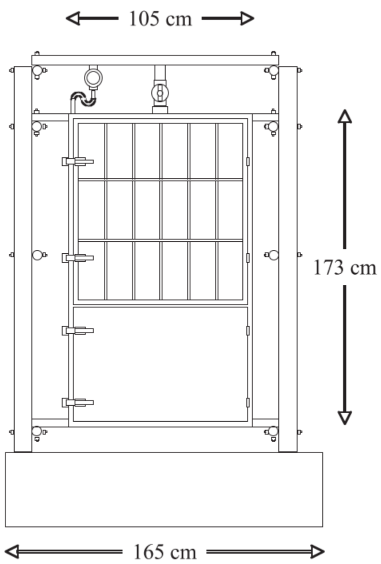


Fig. 2. Front view of the head cage and digestion stall

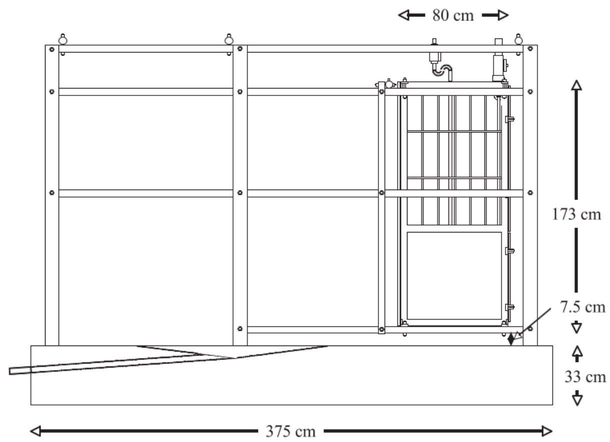


Fig. 3. Side view of the head cage and digestion stall

Fig. 4. Headbox with hood

Fig. 5 . Feed bunk and automatic water bowl

Fig. 6. Vortex blowers

Fig. 7. Flow meter and main air tube

Fig.8a. Pump for air from the main air flow (12 L/min)

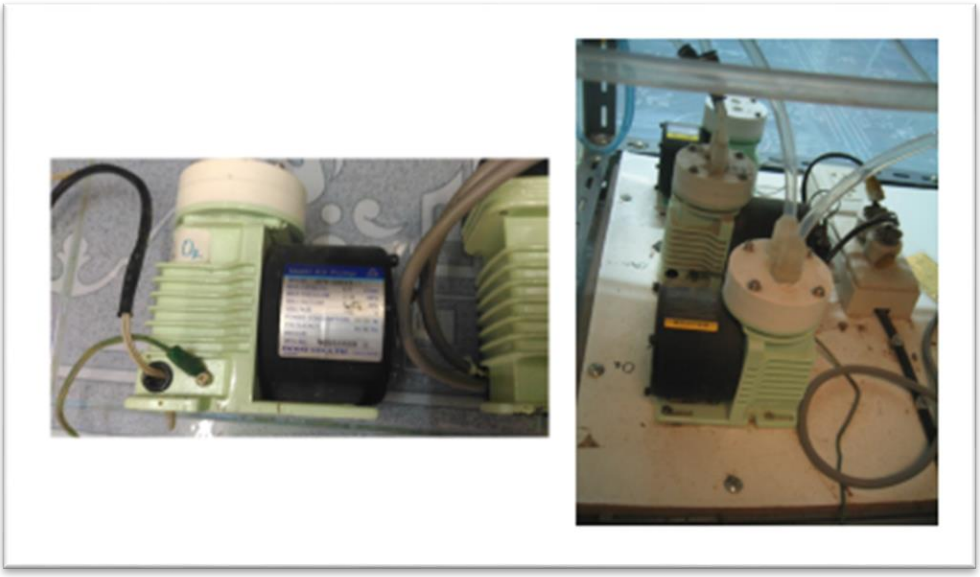


Fig. 8b. Vacuum pump for O_2, CO_2 and CH_4 gas sampling (flow rate 7, 5 and 5 L/min)

Fig. 9. Air cooling dryer

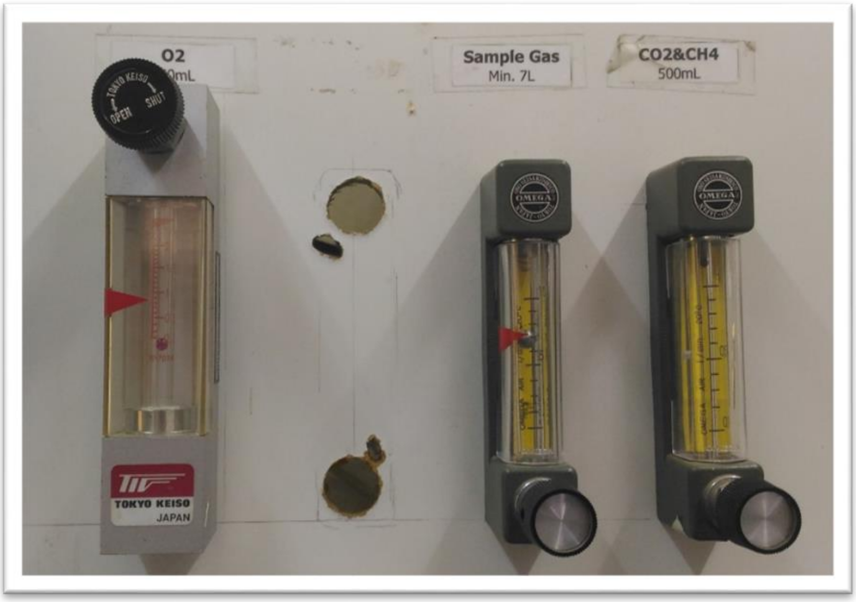


Fig.10. Flow meter with needle valve

Fig.11 Gas analyzers (Carbon dioxide, Methane and Oxygen)

Fig.12. Auto gas sampling unit

Fig.13. Dust filter

Fig.14. Polymer membrane filter

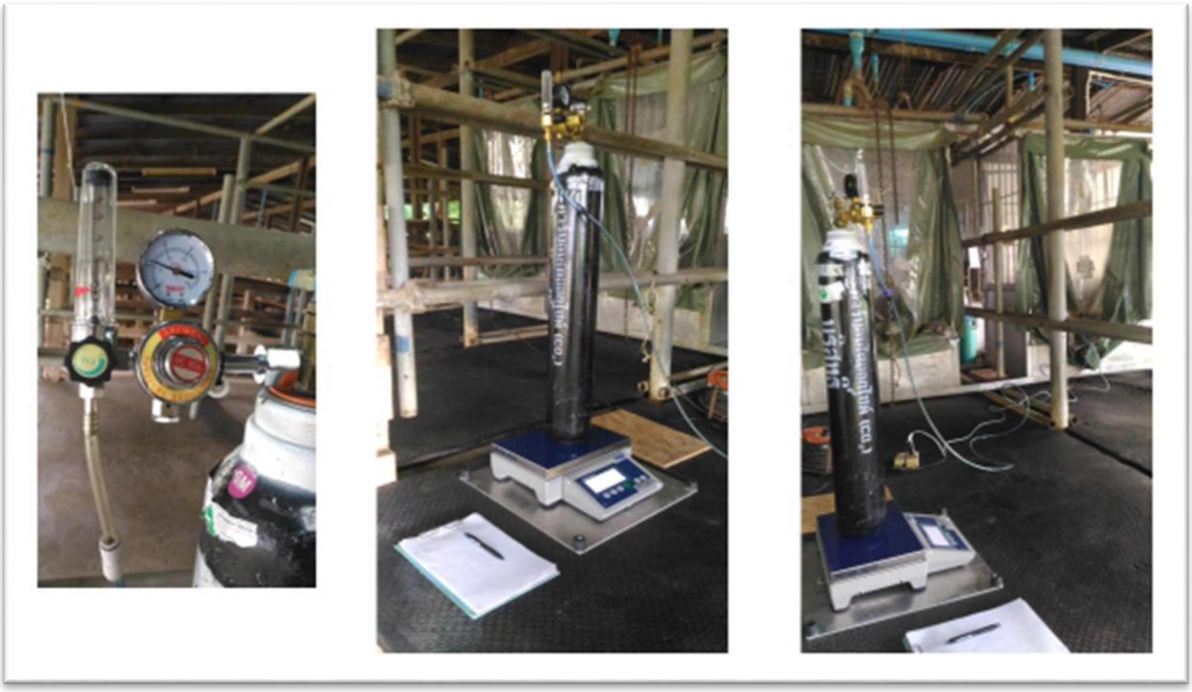


Fig.15. Recovery test

Location: Faculty of Agriculture, National University of Laos, Vientiane capital, Lao PDR.

Scientist Responsible: Assoc. Prof. Dr. Viengsakoun Napasirth
Email: v.napasirth@nuol.edu.la
Description of facility: NUOL's Respiratory headbox facility comprise two parts as: • Feed bunk adequate to feed and record intake of 18 cattle individually. • Four (4) headboxes with the fix air flow, gas sampling system and associated metabolic crates for collection of faeces and manure.
Principle of operation: *Summary:* The four headboxes all operate independently. Air is continuously drawn from the top of each headbox into an exhaust pipe then through an in-line recording air flow meter and into a side-channel blower that draws the air then voids it into the atmosphere. As air is drawn out the exhaust pipe from the headbox, fresh ambient air is drawn into the headbox around the loose-fitting shroud. A continuous subsampling of exhaust and ambient gas into Tedlar bags is made using miniature peristaltic pumps. Each bag of subsampled gas (1 per headbox plus one ambient air bag) is subsequently analysed by a multi-gas analyser. System details are as below: *Headbox details:* 90 L x 80 W x 110 H cm box, raised 30 cm off the ground. Box is made of welded steel screened with 6mm thick polycarbonate. Door is secured by 3 'butchers latches' and has a foam strip between door and box to ensure an airtight seal. The shroud dimensions are as shown in Photo 1. *Air flow:* Air is drawn through each headbox by a side channel of ring blowers (Ventex 2RB010-7AA11), at a rate of 500L/minute. This is the maximum flow possible with this pump. Flow rate is varied by a 'T-piece' fitted into the pipe between the flow meter and the ring blower, with a gate valve on the stem of the 'T' that is open to the atmosphere. By opening this gate vale, ambient air is allowed into the blower, reducing the amount of air being drawn from the headbox & through the flow meter. The flow meter (ATZTA TBX - Aichi Tokei) has a self-contained battery with 7-year life. The display on the in-line meter is recorded at the start and end of the 23h measurement period and divided by time elapsed to calculate flow rate. *Air Temperature and Pressure:* Measuring the temperature and pressure of air before going through the flow meter is done by inserting a hand-held temperature and pressure gauge (Barometric Pressure Gauge PCE-THB 38-ICA incl.) into a sampling port in front of the flow meter. Temperature and pressure are read 3 times per day and the values written down in the experiment log sheets. Typically, pressure is only 3-6kPa less than the atmospheric pressure (approx. 100kPa depending on altitude). *Air Sampling:* A continuous sample of air over 23h from when the chamber doors are closed is collected into a pre-evacuated 5L Tedlar bag. The sample bag is left sealed and connected via tygon tubing to a miniature peristaltic pump (LONGER Peristaltic Pump Miniature BQ50-1J) which is adjusted to pump at 2-3 mL air/minute. The bag is opened and the pump turned

on when the headbox door is closed. The bag is pulled closed to seal 23h later and taken for immediate analysis.

Gas analysis:

The gas is analysed using an Aquagas GA40T+ multigas analyser, that reports O2, CO2, and CH4 gas levels directly on-screen. It is not linked to a computer. The analyser draws approximately 1.5L gas sample/minute and is used as below:

- Analysis is done in a well-ventilated room well away from cattle or other sources of methane (this is so the background CH4 level is truly 0 ppm).
- The analyser is allowed to warm up for at least one hour before use.
- The 'Zero Standard' (Pure N2) and the calibration/span gas (CH4 in N2) are run through the analyser. They are transferred into evacuated Tedlar gas bags directly from the cylinder and then the gas analyser draws sample (1.5L/m,in) from the Tedlar bag into the gas analyser.
  The expected O2, CO2 and CH4 concentrations are written down, along with the observed concentrations.
- Look at the calibration bag results ~ was it within 10% of the value shown in the cylinder itself? If it is <10% different then we can correct all the gas bag readings by whatever the % difference is. So if the calibration gas was meant to be 1,000 ppm but we read it as 950ppm, the value is 5% low, so we would multiply up all the other CH4 ppm values by 5% before we use them. If the reading is more than 10% different than the stated concentration on the cylinder certificate, then you should recalibrate the GA40T+ analyser (or any other analyser). This is a complicated procedure for the GA40T+.
- Each of the 5 Tedlar bags (background or ambient bag + 4 headbox bags) are then sequentially sampled for 15-30 seconds – the reading should have stabilised on the analyser display by then. Write down or print the results for all 3 gases.
- Then repeat the measurement of each bag, recording the concentrations shown 40s after connecting each bag.
- The Zero Standard and the calibration gas bags are then repeated.

Gas recovery procedure:

A short-term 3min recovery of tracer gas (CO2) delivered up the exhaust outlet is used to calculate gas recovery. CO2 is released from a small cylinder at a fixed rate of 10-15g/min using a regulator with a rotameter. The cylinder is placed on an electric scale reporting to 1g and every 30s the cylinder weight and the CO2 in the exhaust air is recorded. Weight loss from the (small) cylinder is multiplied by the flow rate to work out the expected (100% recovery) increase in CO2 level expected and this is compared with the ppm of CO2 observed.

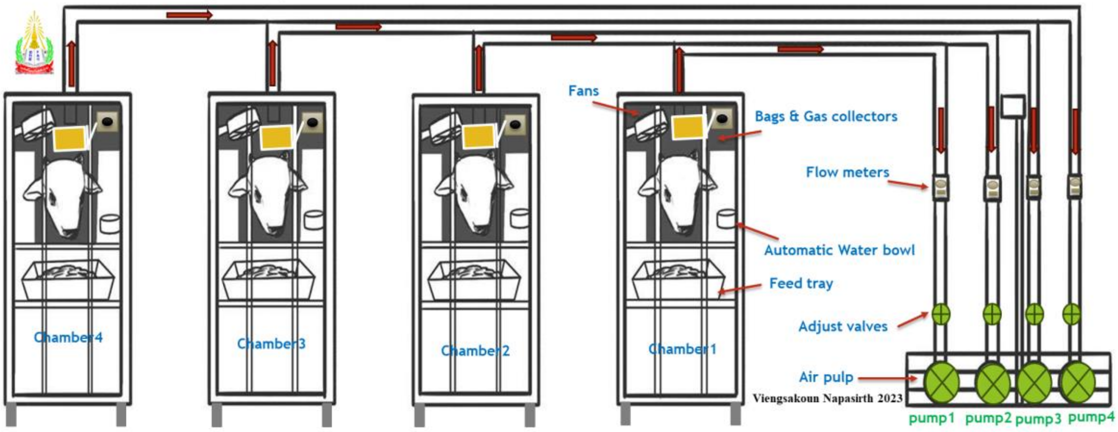
Photo of NUOL's Respiratory headbox facility comprise two parts as below:

Photo of respiratory headbox chambers metabolic crates system in National University of Laos:
Front of respiratory headbox chambers metabolic crates

Back of respiratory headbox chambers metabolic crates, showing adjustable back rails and the wooden loading ramp in place (below).

Sketch with measurements of headbox – so somebody can copy design

Respiratory Headbox chamber metabolic crates in FAG-NUOL Lao PDR.

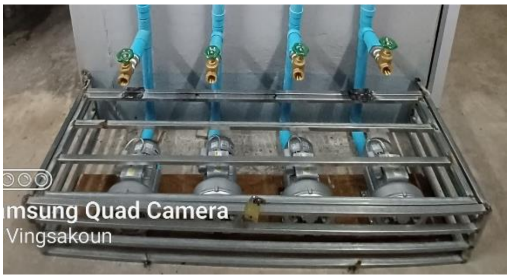


Manufacturer's template for neck shroud construction

Front headbox chamber Back of headbox
chamber

Photo of airflow components

- Blower fan and any air bleed-in valves



- Flow meters

Photo of gas collection, analyser & and filter/dryer components

Photo of gas recovery in action