9 ANIMAL MANAGEMENT AND FEED INTAKE

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Methane (CH_4) is derived from anaerobic digestion of feed, mainly in the rumen, and CH_4 production is largely determined by the amount of feed eaten. When calculating CH_4 yield (CH_4/unit of feed intake), an accurate measure of intake is just as important as an accurate measure of CH_4. The SF_6 technique is important, because it enables CH_4 production to be measured in environments that are typical of commercial husbandry.

The SF_6 technique allows CH_4 measurement from unrestrained animals, which therefore exhibit 'normal' behaviour such as grazing. But in many instances, the technique is used with animals confined in pens or metabolism crates. Because most of the world's ruminants graze outdoors, measurements made from grazing animals may best represent the population of animals managed on farms. They choose their diet, and when to eat, ruminate and rest. The importance of grazing behaviour – and grazing regimens – should not be underestimated in terms of production, digestion and possibly, CH_4 emissions.

The alternative 'cut and carry' feeding of confined or restrained animals provides feed at times determined by experimental protocols, often with higher intakes compared to competitive grazing. Forage is cut to a defined length, and intakes are rapid. Nevertheless, if intake measurement is an important aspect of an experimental protocol, feeding measured amounts, and collecting uneaten feed (refusals, orts) is the only way to ensure an accurate measurement.

The SF_6 technique applies to different ruminant species under a wide range of circumstances, but in practice, most measurements have been confined to sheep, cattle and some deer. Few measurements have been made with males (other than

castrates) or animals that have not had frequent contact with humans. The SF_6 method involves frequent – often daily – handling during the measurement period: fitting halters and gas collection canisters and ensuring the tubing, or 'plumbing', remains intact.

It is important that animals are reasonably docile. There is nothing to be gained from trying to measure CH_4 production from angry animals, other than poor data, broken equipment and injuries to humans and animals. Animals should be sufficiently calm for equipment to be attached and changed without headstocks, because neck restraints prevent neck yokes being fitted, and damage can occur when the animal is released. *Bos indicus* is naturally more agitated and averse to human contact, and typically demands two to three months of careful care during domestication. Young animals can become very docile following domestication, but it is best to identify aggressive animals and exclude them prior to training to ensure success. This applies to all species and breeds.

The experimental objective should determine both the choice of protocol – whether SF_6 or another method should be used for measuring CH_4 – and the requirements for intake measurement (Table 9.1).

The importance of the objective cannot be overstated, especially when it defines grazing vs. indoor feeding. Are intake measurements really necessary? Could they be predicted from energy requirements for maintenance and measured production? How accurate should the measurements be? Indigestible markers, faecal 'grab' samples or pre- and post-grazing pasture cuts can give an indication of intakes, and avoid the need for cut–and-carry, which means intakes can be measured indoors. However, there is a risk of under-estimating CH_4 yields if predicted intakes are used, because any reduction in actual intake (in response to the measuring equipment) will lower CH_4 yields. Of course, these considerations only apply to animals grazed outdoors.

The SF_6 technique has been used to estimate CH_4 production in countries such as Australia, Argentina, Brazil, Canada, China, France, Ireland, New Zealand, United Kingdom and the United States. When protocols have been followed, the data have been assumed to be representative of the actual emissions. Indeed, emissions calculated from the SF_6 method have been compared with respiration chambers in cattle (McGinn et al., 2006; Grainger et al., 2007; Münoz et al., 2012) and sheep (Hammond et al., 2009) with good agreement.

However, when sufficient data became available, Vlaming et al. (2005; 2007) demonstrated a positive relationship between SF_6 permeation rate and estimates of CH_4 production, and this warrants further investigation. Other concerns with this technique were highlighted when low CH_4 yields (g/kg dry matter (DM) intakes) measured from sheep fed either fresh white clover (Trifoliun repens) (Krause; unpublished) (14-16 g/kg DM intake or chicory (Cichorium intybus) (Waghorn et al., 2002) (16.2 g/kg DM intake) were reassessed using respiration chambers. Chamber measurements showed CH_4 yields from sheep fed white clover were 19.8-27.1 g/kg DM intake (Hammond et al., 2011), and 22.8 g/kg DMI from chicory (Sun et al., 2011).

Intake level may have accounted for some of the discrepancies between SF_6 and chamber values (Hammond et al., 2013), but much of the difference between measurements methods remains unexplained.

This chapter considers three aspects of measurement in farm animals; animal handling and management, intake measurement, expression of CH_4 and diet composition.

9.1 Animal handling and management

The SF_6 technique requires eructated and respired gas to be collected from near the nostrils, and the sample stored in evacuated canisters that are changed when they are between 50 and 70% full. The sampling tube must be attached to the animal in a way that maintains its position near the nostrils at all times, whether eating, drinking, scratching or any other activity. Usually, it is connected to a halter securely fastened to the animals head. A 'nose flap' or similar may need to be fitted to ensure the sampling tube is located appropriately, and the tube should have two openings in the shape of a 'y', to minimise the chance of water blocking the capillary tube when the animal is either drinking or grazing wet pasture. The actual position of the sampling tube is not important, provided the collected gas concentrations are about 10 times their concentration in background air.

Halters are the standard method of attaching the sampling tube, and any well-constructed (i.e., several adjustable straps) version is suitable, provided it fits firmly without rubbing. Halters for cattle seem to fit easily and well, but sheep can be more difficult, depending on wool length and halter design. It is probably easier to work with sheep that have comparatively little wool around their head and neck, which means the halter can be positioned easily and securely. Halters may be fitted one or two days before sample collecting, so animals become accustomed to them and to people. It is probably best to commence measurements from all animals in a trial at a similar time.

The **canister** design (Chapter 5) may affect animal handling and operator safety. Early trials in New Zealand placed 'training' neck canisters on sheep and cattle, so they became accustomed to the equipment before measurements commenced. In Brazil, most trials with cattle use training halters and canisters (without tubes and connections) for at least four days before the collection period. The need for training halters can also be assessed by measuring CH_4 production over sequential collection days. Brazilian work has not shown any changes in emissions measured over five days following a four-day training period.

The **animal handling requirements** for collection canisters are similar to those for fitting halters, but care is essential to make sure the animals are not harmed by the equipment. Girth straps holding back-mounted canisters can cut and abrade behind the front legs, and the canisters themselves can damage the skin over the spine. These require straps around the brisket, in addition to the girth strap, rather than very tight girth straps alone. The use of shoulder and saddle collection canisters has been restricted in some centres because careless or poor fitting by some operators caused rubbing and pressure damage.

On housed animals, collection canisters must sometimes be mounted in alternative ways: for example, neck-mounted yokes are not appropriate when animals are eating from Calan gates, Grow Safe or any facility with a narrow access point to feed. Individual animals held in metabolism stalls may have the collection canister mounted off the animal, in which case the tubing connecting to the halter should be positioned so the animal cannot chew it. This may be achieved if it is attached behind the head, or between the shoulder blades, and if the tubing is supported by elastic (7-10 mm, used in clothing) it will remain out of reach when the animal stands or lies down.

Other systems include mounting under the jaw, attached to the halter, but these may interfere with grazing. Animal care and welfare is essential from both an ethical perspective and because an ill-treated animal will yield poor quality information.

When **fitting halters, harnesses and canisters**, it is preferable that animals are sufficiently docile that head and/or neck restraints (or crushes) are not necessary. In our experience, it is better to spend time working with the animals for days or weeks before the measurements, so they become accustomed to handling, rather than impose halters and other collection equipment on stressed sheep, cattle or deer.

We should aim for 100% success with collections, and if the collection rate is less than 80%, something is wrong with the equipment installation, animal training or operators. Problems arise from loose tubing, stressed animals and inadequately trained personnel fitting and changing equipment incorrectly. Experienced personnel will observe, identify and remedy potential problems: are connectors fitted properly? Are tubes intact, stretched or broken? The vacuum in the canister must be checked when placed on, and removed from, the animal, and collections should be avoided in very wet conditions because tubing will block. Attention to detail will improve the quantity and quality of samples obtained, but often, only 70% of collections (with appropriate vacuum) from grazing animals are successful. In this situation, it is important to allow extra days for measurement, so as to obtain sufficient robust data.

Experimental animals should be split into **groups** of less than 15, to minimise equipment damage when they are bought into the yards, and to maximise their grazing time – large groups take time to process, so there is less time available for grazing. Keeping groups small, and having enough operators on hand, will ensure the rapid exchange of canisters. Keep spare halters and canisters handy should any need replacing.

It is also important to consider herd hierarchy: mixing animals from different herds immediately before the collection period might see equipment damaged as animals establish a new dominance relationship. When animals alter their behaviour in this way, they probably eat less and spend more time and energy on social interactions, which means data quality can be compromised.

Any **reduction in intakes** attributed to the equipment will affect measurements and interpretation, and this could prejudice data accuracy – especially where intakes were calculated or estimated. Most sheep and dairy cows have little problem with

measuring equipment, but young animals appear to reduce their intakes – for one or two days, at least – when measuring apparatus is placed on them. However, once accustomed to the halter, the canister appears to have minor effects on behaviour of mature sheep and cattle. This means measurements may be taken from the time the canister was fitted. Fitting halters takes time, so the halter may be fitted one or two days prior to placing the canister on the animal.

Determining an animal's appropriate adjustment period must be made with specific experimental situations in mind. Any impact from the canister can be assessed by comparing emissions on day one with those on subsequent measurement days.

The **optimal number of collection days** does not appear to have been determined. In Brazil, five days of measurements from cattle are recommended – including additional collection days if some data are discarded – and this has resulted in consistent emissions without trends over the collection period. Measurements from cattle in New Zealand have occasionally shown oscillations in daily emissions (a two-day cycle). The cause has not been identified, but it could be the intermittent equilibration of SF_6 in rumen head-space gas. Defensible measures of CH_4 made using SF_6 must be undertaken over at least four days, because the data will be more representative than from shorter measurement periods, and any trends over time can be recognised over a period of four or more days. We suggest a minimum of four days of collection – three days is inadequate.

9.2 Determining feed intake

In most situations, feed intakes will be an important component of CH_4 research, and this information may be fundamental to the interpretation of results. Intake measurements are easily achieved with indoor trials, because feed offered and refused by individual animals can be measured. Even under these conditions, however, it is important that researchers are aware that at the start of a trial, the CH_4 is derived from material eaten previously, and the feed consumed at its conclusion is contributing CH_4 that will not be measured. It is therefore worthwhile measuring intakes for a few days prior to CH_4 measurements, especially to be sure there are no changes (most likely a decline) associated with measurements. A consistent level of intake and feed type, and a prolonged measurement period (four to five days) will improve the accuracy of any determinations. Consideration may also need to be given to rumen adaptation to dietary change, especially when the same animal is used in a crossover or Latin square design.

Intakes of grazing ruminants are difficult to estimate, and impossible to measure accurately. It is also difficult to assess the accuracy of grazing measurements. 'Obviously' incorrect intakes can be easily identified: of more concern are assumptions that most measurements are 'acceptable.'

The use of tables and equations to estimate intakes of animals in *short term* grazing trials is equally unacceptable, because daily variation in actual intakes is too great, and

a 10% overestimate of actual intakes could create a 'significant' treatment reduction in yield, when in reality, there was none.

Researchers cannot assess the accuracy of intake calculations derived from feed requirements based on changes in body weight and production. Ruminant weight is notoriously variable, because the rumen digesta accounts for 10-20% of body weight (Archer et al., 1997; Waghorn 2002) and can vary substantially within, and between, days. In addition, mobilisation and accretion of body tissue can affect feed energy associated with milk production in lactating animals, so intakes cannot be calculated from productivity alone. In beef cattle, the muscle and fat deposition rates alter during growth, so it is also hard to calculate the efficiency of feed energy use. To address these challenges, researchers must define the experimental objective carefully. Once defined, the appropriate experimental protocols can be applied.

If intake measurements are required, the experimental design must take into account the need for either 'accurate' or 'natural' (outdoor) conditions. Accuracy may be achieved by indoor feeding, but this will not be 'natural' for a grazing animal. 'Natural' may be indoor feeding in some environments, but with grazing animals, the intakes will be affected by the amount and accessibility of feed offered, weather, animal efficiency (residual feed intake), reproductive cycle (oestrus), social status in the group, effect of CH_4 measuring equipment and management. Furthermore, all these are affected by physiological state (Table 9.1).

When expressing CH_4 production in terms of intake (i.e., yield), equal value must be placed on the accuracy with which *both* measurements are made. Table 9.1 lists some positive and negative points relating to indoor feeding, grazing and calculated measurements of intake. When designing experiments intended to express CH_4 in terms of feed eaten, researchers must consider many factors. The points in the Table will not be expanded here, but the appropriate technique will balance the risks with the research objectives.

A realistic risk assessment must be made prior to measurements, rather than optimistically assuming the findings will be acceptable. Of most concern is the acceptance of data when it is probably flawed (e.g., estimates of intake), in order to achieve an outcome (publication), without appropriate consideration of the accuracy of the data. Unfortunately, this is too common, and has lead to misleading and incorrect conclusions in CH_4 and all research. We reiterate: the measurements must be driven by the objective, and in some situations, intake may not be necessary when evaluating a treatment on CH_4 emissions: for instance, when testing mitigants of CH_4, or determining emissions intensity (Ei; emissions/production).

If the experimental objective is to reduce CH_4 emissions while maintaining production, it may be possible to simply measure CH_4 from farmed animals. If done over an appropriate period, without experimental bias (for example live-weight gain and loss, in dairy cows over a lactation), good information can be achieved about emissions, production and therefore, Ei. This is emissions/production and, in a hungry world, may be a more sensible measure of greenhouse gas emissions than yield.

Table 9.1: Positive and negative aspects of intake measurement from housed, grazed and estimated values.

Indoor feeding

Positives

Accurate weights of feed offered and refused.

Accurate sampling of feed offered and refused to determine dry matter percentage and composition.

Accurate measurement of feed eaten.

Appropriate management for animals raised indoors (e.g., dairy tie-stall, free-stall).

Concerns

None, if data relate to indoor management systems, and intakes are recorded accurately.

Intakes and digestion can be affected by timing/feeding frequency, even when feed is always available.

The feeding pattern will be determined by the feeding regimen.

Negatives

Forage – and to a lesser extent, silage composition – changes after it is cut or removed from storage, raising the risk of heating and spoilage.

Indoor forage feeding is not representative of a grazing environment because:
Forage is cut once or twice a day.

Forage is harvested to a predetermined height; it is often longer (and more mature) than grazed forage, to make it easier to harvest.

Intakes are likely to exceed that at grazing because of *ad libitum* availability.

Digestion will differ from grazed forage because cutting length is pre-determined and less chewing (cell damage) may be required, compared to grazed forage.

Animal selection of plant species and plant parts is limited.

Interaction with peers and time for other activities are avoided/compromised.

Hours of light/dark are altered.

Grazing

Positives

Grazing represents the 'real world' under which most ruminants exist in many environments and countries.

Intakes are usually limited through availability and competition with other animals.

Forage quality varies, but animals are often able to choose a variety of components in their diet.

Concerns
Intakes vary with feed availability, competition, specific paddocks, animal management, drive to feed, etc.

Composition of diet will differ for individuals, and during the day. Under rotational grazing where new feed is given once or twice daily to achieve high forage utilisation (e.g., pasture-fed dairy cows), diet quality will diminish during the day.

In slower rotations, where animals stay in the same paddock for three to five days, the changes in forage composition, availability, and grazing behaviour are also likely to affect CH_4 emissions. With slow rotations, the periods of CH_4 measurement may be adjusted to fit the experimental objectives (e.g. three to five days in a five-day rotation).

Digestion and digestibility will be affected by diet composition, eating pattern, intake level and behaviour.

Negatives
Intakes are usually limited through competition with other animals or availability and forage quality.

No satisfactory method for estimating feed intakes.

Pre- and post-grazing pasture cuts have moderate accuracy and can be appropriate for estimating group intakes.

Measuring faecal output (with an indigestible marker or collection bag and harness) requires knowledge of digestibility to calculate intakes, but digestibility varies substantially between individuals; values may be more defensible for groups than individuals.

Use of faecal collection bags risks losing faeces, underestimating intake and increasing estimates of CH_4 yield.

The alkane (plant cuticular wax) method has an advantage over external (indigestible) markers, because variation between individuals in digestibility is accounted for in the calculations. This technique is based on faecal recovery of plant waxes and a synthetic wax (usually an even chain length) administered daily to the animals. For the method to provide accurate (reliable) data, *researchers need to know the alkane content (and type) of the diet eaten, and the recovery (indigestibility) of both the plant and*

administered alkane must be the same, but In a mixed sward, it is not possible to determine the alkane concentration in forage eaten, because values differ between plant species, and individual animals vary in their dietary choice.
Measures of faecal recovery of alkane waxes show differences between plant and administered waxes (administered are usually higher than plant waxes).

Alkane technology is claimed to be efficacious when monocultures are grazed, but this is difficult to demonstrate.

When feed intake is estimated using external markers such as alkanes, chromium oxide and titanium dioxide, animals need to be dosed twice daily, usually for 12 days – seven days to reach steady state, followed by five days with twice-daily faecal collection. Depending on animals and circumstances, it may be best to undertake CH_4 and intake measurements separately; especially with animals such as sheep or beef cattle, which are unaccustomed to routine handling. However, both can be done simultaneously with dairy cows that are handled every day.

Calculated intakes

Positives
A value is generated, and over a period of several weeks this is likely to be a representative group mean.

Concerns
The number may have little relevance to intakes of animals fitted with CH_4 sampling apparatus, especially in short term trials.

Negatives
Feed intakes are calculated from existing tables of energy requirements, which are based on experimental data collected under situations when intakes could be measured. An average value is then derived for animals with a defined weight and productivity. Some systems (e.g., the Australian Research Council standards, 1990) take the environment into account when deriving the values, but none can take into account differences between individuals (residual feed intake, or RFI).

The energy requirements are usually based on production, live weight and live weight change, and must be measured in conjunction with CH_4 measurements. These data cannot be determined accurately in short-term trials, nor can the metabolisable energy content of the feed eaten.

Calculated intakes mean little in the short term, and values for young animals fitted with CH_4 collection apparatus will inevitably be overestimates of actual intakes. This situation would underestimate actual yields.

9.3 Expressing methane and measuring diet composition

When intake is measured, should CH_4 yield be expressed as dry matter (DM), organic matter (OM), digestible DM, or energy (gross, digestible, metabolisable or net)?

Despite current and past protocols, it is illogical to express CH_4 on either a gross energy (GE) or DM basis, because they do not account for variations in feed quality, nor for the source of CH_4, which is digested feed. Production-targeted feeding is based on diet composition and quality, and where energy is first limiting for production, diets are assessed on the basis of available energy content for maintenance and production (metabolisable energy, or ME).

Adding fat – or reducing ash – elevates a diet's GE, and most forages have a value of about 18.4 MJ/kg DM, even though there may be a two-fold range in the feeding value for production. For example, a grain-based diet will result in much higher production, and efficiency of production (daily gain/daily feed intake) than a diet of fibrous forages. Historically, CH_4 has been expressed on a GE basis, and more recently on a DM basis, for inventory. Expression on a DM basis may be justified if feed requirements have been based on DM requirements, but in reality, this is dated and inappropriate. Expressing CH_4 emission based on gross feed intake may be acceptable for emissions inventory purposes, but mitigation research needs to evaluated on a more meaningful basis.

Expression in terms of organic matter is logical, because the CH_4 does not originate from the ash component of feed. However, it is not much more useful than DM, especially as ash accounts for between 7 and 10% of DM in most feedstuffs. When energy is first limiting for production – as with good quality temperate pasture species – it would be sensible to express CH_4 in terms of ME, because this is the basis of determining either the feed required to achieve a predetermined level of production, or predicting the production likely from a set ME intake.

So, ME may appear a logical way to express CH_4 emissions, but a feed's ME varies with intake, and the efficiency of use for production is affected by diet composition (Waghorn, 2007). ME is not a constant, and is usually predicted rather than measured.

There is some logic in expressing CH_4 on the basis of material digested, but only ruminal and hind-gut digestion contributes CH_4. Digestibility varies with intake, feed type and individual animal, and some reports have suggested a poor relationship between CH_4 yield and digestibility (Johnson and Johnson, 1995; Hammond et al, 2013).

These variables highlight some of the factors that should be considered when designing an experiment, but there is no right or wrong method of expression. The important thing is that measurements of feed intake and feed composition are accurate and repeatable.

One of the most difficult – though often unrecognised – challenges faced by researchers is measuring forage DM content and composition, especially of wet

forages fed indoors. This could be illustrated by a feeding trial with cattle in a barn situation, where 5 t of wet pasture is offered daily, but less than 2 kg might be used to determine the DM percentage of material offered, and less than a gram is used for analysis. The problem is greater still with very moist forages, and is made worse when the material contains a range of plant species. For example, if the feed offered is about 12% DM, then an error of ± 0.5 of a percentage unit (11.5 to 12.5% DM) represents an 8.3% variation in feed offered.

Errors in feed DM determination can be minimised by taking several samples, then drying 'representative' samples (200 g wet weight) when new feed is given, and in triplicate. It is important that samples represent the material offered, and that they do not dry out prior to oven drying. They should be placed in a plastic bag, held at 4^{o}C, and when removed for drying, sub-sampling and weighing, this should be done quickly.

The problem is less important with refusals (orts), especially if these represent 10-15% of material offered, because the error can only be 10-15% of that associated with the feed offered. Sampling for analysis represents another challenge, and sometimes grinding a large, rather than small, sample will lessen errors associated with sub-sampling. When material is ground in a Wiley mill, there is always some residue remaining in the mill. This is inevitably stalky material, so grinding in effect lowers the fibre content of the sample submitted for analysis. Some labs do not clean the mill between grinding samples of similar material, in an effort to maintain representative material.

The assays themselves are really the prerogative of the researcher, and may be based on wet chemistry or near infrared spectroscopy (NIRS), but it is imperative that all samples are prepared in accordance with analytical requirements. Samples used for DM determination (e.g., dried at 105^{o}C for 24h) will not be suitable for chemical analyses. It is helpful to keep a spare sample (in the dark) so additional analyses can be carried out, if needed.

9.4 References

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