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Respiratory chamber facility

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Introduction

This guideline will highlight the key steps required when measuring gas exchange of cattle for the estimation of methane emission and heat production (by estimating the relation of emitted CO_2 and CH_4 to the consumed O_2) via a respiration chamber (RC). The authors acknowledge the variation in RC design in different experimental units, and therefore, the mentioned steps within this guideline are common and essential for all. Note that a CO_2 and/or CH_4 recovery test from the RC should be performed before measuring methane or heat production, and that this guideline is presented in a separate chapter. The Animal Trait Ontology (ATOL) and Environment Ontology (EOL) for Livestock numbers linked with this guideline are: **ATOL_0001390, ATOL_0001391, ATOL_0000772, ATOL_0001529, ATOL_0000837, ATOL_0000835, ATOL_0000359, ATOL_0000363, ATOL_0000351, ATOL_0000239, ATOL_0001393, ATOL_0001491, ATOL_0001518, EOL_0001905, EOL_0001618, EOL_0000178, EOL_00001712, EOL_0001684, EOL_0000067** and **EOL_0000164** (for the complete list of ATOL, please visit https://www.atol-ontology.com/en/erter-2/).

A – Prior to start of an RC experiment

1. If the system has more than one chamber, it should be stated in the experimental plan how animals/treatments are allocated to the different chambers, i.e. different treatments represented in different chambers.
2. The animal must be adapted to the RC conditions of restraint and confinement prior to the start of the first sampling period. Duration of acclimatizing in order to reduce the stress on the animal usually depends on the individual's suseptibility to stress and the handling of the animal prior and during transportation. For animals not accustomed to being restrained, the experimenter should record the adaption procedure. Dry matter intake, standing and lying behaviour before and after restraint are good indices of the adaptation of animals. Therefore, the measurement of dry matter intake before, during, and after the chamber stay is recommended. For some cases, adaptation of milking cows to the RC's milking system may be required.
3. The body weight of the animal should be measured before transfer into the RC. Measurements of bodyweight should always be made at the same time of day, relative to feeding and milking.

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4. Unless the intention of study is to investigate the impact of diet change or phase feeding, the ration provided to the animal in the RC should be the same as fed at least during the previous 14 d (minimum adaptation) and should not change during the RC measurement phase.
5. Animals are normally restrained within the RC in tie stall system. The tie stall system should permit 'normal' movement with respect to standing, lying and feeding activity. If nitrogen (N) balance is to be measured simultaneously then the tethering system should restrict rearward mobility. The lying surface should be comfortable, incorporating a clean mat or mattress and if the system or experimental diet under test allows, bedding should be used in the lying area (e.g. wood shavings).
6. The cleanliness and function of the drinking trough and – if available – the water intake measuring system should be checked and calibrated.
7. The facility should have a checklist of important technical issues to be looked over prior to the start of an experiment, e.g., air circulating set point, dust filter cleanliness, gas sampling system (pumps, filters, flow, pressure and humidity controls), gas analysers (zero and span point check and range adjustments in case of abnormal drifts), emergency systems, climate set points (temperature, humidity, lighting regime), air conditioning systems and ventilation and air pressure set points. A logbook with the result of the checks should be filed out to ensure problems can be tracked.
8. Feed bins in the chamber should be clean and empty and the feed bin scales (if fitted) should be examined and tared to zero.
9. Gas analyzers must be checked regularly (minimum twice weekly) using calibration gases of known composition (zero and span). Adjustments are to be done only when the analyser's zero or span is significantly out of normal drift range. Data from checks can be used to calculate correction lines that vary in time (assuming a linear drift of zero and span values in time).
10. For lactating animals, the milking system should be prepared and the vaccum control tested at least yearly. If a balance is used for weighing the milk, it should be calibrated prior to the beginning of the trial. The use of suitable calibration weights on all weghing balances (animal, feed, milk) is highly recommended during the experiment.

B – RC experiment

1. The operatives should ensure that the RC doors are secured, setpoint differential air pressure is reached and all relevant equipment and instruments are turned on and functioning properly.
2. The chamber air handling system must have reached equilibratium (flow, temperature, humidity) before measurement begins. If all climate and ventilation data is measured and logged continously and suitable models for calculation of gas exchange are used, then masurement can begin from the moment the door(s) is (are) closed, and setpoint differential air pressure is reached.
3. Animals should be fed at the same time each day. Feed should be prepared and weighed in advance and fed after the gas exchange measurement for the day has started. If wanted, the logging can be automated via simple switches on all doors, which would be connected to a data acquisition system.
4. In systems where doors must be opened for milking or cleaning etc., opening and closing times and the reason for opening should be recorded in a logbook.
5. When an air-lock is constructed within the RC, staff members should load the feed bins and milk animals by walking through the air-locks and observing the requirements for air equilibration. In RC systems without an air-lock, operatives should open the RC access door for as short a time as possible and close the door while carrying out feeding and milking procedures. When exiting the RC, the access door should be opned for as brief a period as possible. Avoid leaving access doors open for extended periods of time (see the recommendations stated above).
6. The airflow in-or-out of the chamber should be set in a range, which allows the gas analytical system to record the respired gas concentration in the calibrated range. This range depends on the sensitivity of the gas analysers, the body weight and the physiological stage of the animal.
7. Data acquisition during an RC experiment should be conducted for at least 48 h and ideally for 96 h to account for daily fluctuations in output. Where faeces and urine are to be collected for energy partition or N balance studies, animals should be housed in the RC for at least 5 consecutive days (the length of the collection period can vary, depending on the frequency of defecating/urinating). The maximal duration an animal can be held in the RC may be dependent on national animal ethics regulations.
8. During the RC measurement phase, animals should be milked in the RC 2–3 times per d at the same time each day and ideally with equal time distances between each milking. It is recommend

to measure milk yield and milk composition during RC measurements if methane production is to be related to energy-corrected milk yield (methane intensity). Milk samples should be collected at each milking for the whole sample phase. Thoroughly mixed samples should be stored at 4°C with the addition of an appropriate preservative (e.g. Bronopol) until analysis. If analysis is to be carried out later (>4 d), samples should be stored at −20°C.

9. The timing of feed offered during the experimental period should be consistent, with animals fed at the same time or times in relation to sampling or milking events. Animals can be fed either ad libitum, with 5–10% refusal (on a dry matter basis), or, alternatively, the offered feed level can be calculated based on the average daily dry matter intake during the week prior to sampling, thus providing a daily feed allowance based on actual intake. Feed intake data of the animal during the RC measurement phase should be compared with the intake data prior to the start of measuring phase (during the diet adaptation phase; see point 4, section A).
10. Feed refusals must be weighed daily and recorded. Samples of feed offered and refusals should be collected daily during the sampling phase and kept frozen at −20°C for further processing prior to nutritional analysis. Thawed samples can be pooled, mixed and subsampled then subjected to dry matter, nutritional and gross energy (GE) analysis (see chapter "Feed and water intake ").
11. Animals should be fed the same diet throughout each measurement and sampling phase, avoiding any changes in batch, silo or clamp (unless the nature of the experiment dictates otherwise). A total mixed ration (TMR) is recommended to be offered to the animal (see the feed and water intake measurement chapter). In the case of food sorting or mixing between concentrate and forage, when parital mixed ration is offered, it is necessary to quantify and analyse the sorted uneaten feed (refusals) to account for changes in GE concentration.
12. Daily cleaning of the chambers during the measurement phase should be conducted by the operatives. Cleaning should consist of removing and collecting feed residuals from the bins, scraping the mats and replacing the bedding (if used). If the design of the system allows, the cleaning procedure should be conducted with RC doors closed. The RC doors should only be opened briefly to allow equipment and operatives to acccess the RC.
13. Particularly in chambers without airlock entries, the interpolation method for filling the time gaps in gas measurements due to opening of the RC door should be clearly stated. Operatives should log each opening and closing of the access door of the RC, in order to facilitate correction for these time-points during the final data analysis. If possible, operatives should open the access door of each chamber after the measurements have been made to minimize data lost. If necessary, operatives should consider opening the RC access door prior to feeding, when the gas emissions and oxygen consumption are low. By recording door opening, operatives will be able to examine data for these logged time gaps and compare it to average data from the previous hour, allowing the data to be smoothed if there is a change in gas concentrations. Alternatively, periods during which doors are open and operators are working inside the chambers can be removed from the analysis. Comparing datasets with and without these time gaps could provide an overview of the potential errors. However, as this would only be roughly 2%, in a twice daily feeding/milking setup, errors in the daily average will be very limited.
14. Ensure, all measurement procedures and sample collections (milking, weighing, feed refusals, etc.) are concluded before ending the RC measurement phase and moving the animals out of the RC measurement phase.

C – Post RC experiment

1. Data acquisition can be stopped at the end of the experiment.
2. Pumps, ventilators and – if available – video recording and communication systems, should be switched off. As long as staff works inside a chamber (e.g. for cleaning), it is not recommended to switch of any system for safety reasons.
3. Animals must be weighed immediately after the experiment.
4. The chamber should be cleaned after the experiment and operatives/barn staff must pay attention to those sensors that are sensitive to water and humidity.

D – Calculation of gas exchange from data measured in the respiration chamber

In literature, several calculating methds are described to calculate gas exchange from respiration chamber data. Depending on the chamber design and ventilation setup, these methods could differ considerably [1]. It is recommended that lab/chamber dependent calculations are to be used, also to calculate results from CO_2 and/or CH_4 recovery tests. Diluting air with CO_2 and/or CH_4 in the first instance decreases chamber's O_2 level, apparently because O_2 is consumed. The O_2 consumption can be computed from the recovery tests, indeed this shoud not significantly differ from zero.

References

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