Recommended Protocol for Using the GreenFeed System to Measure Gas Exchange from Cattle as Part of the GHG Quantification Project

25 November 2024

Overview

The Greenhouse Gas (GHG) Research Network is measuring the impact of management practices on nitrous oxide and methane emissions, and soil carbon sequestration. Data will be used by researchers to improve outcome estimates, including through the advancement of models and tools. The GHG Research Network is organized into four sub-teams that target GHG measurements in different agricultural sectors, including Land Emissions, Enteric Methane, Animal Housing and Manure Storage, and Tall Towers.

Each of these four sub-teams has developed GHG measurement protocols to provide technical information on the methods used to measure GHGs and applicable data processing procedures. Protocols outline the method used by the Agricultural Research Service (ARS) for this specific project. Other efforts may use different protocols. The protocols are published to promote dialogue and feedback, and to serve as a reference for other research when applicable. Protocols will be updated as needed. This document is the protocol for the Enteric Methane subteam for using Greenfeed systems to measure gas exchange from ruminant cattle.

Measuring gas exchange from ruminant livestock represents an important aspect of research. According to the sixth assessment report by the Intergovernmental Panel on Climate Change (IPCC), enteric methane (CH_4) represents 5.1% of global anthropogenic greenhouse gases (GHG; Dhakal et al., 2023). The latest estimate by the U.S. Environmental Protection Agency (EPA) reports enteric CH_4 representing 3% of total U.S. GHG emissions (EPA, 2024). To meet governmental agreements and commitments (UNFCCC, 2016; The White House, 2021), experiments to quantify and mitigate enteric CH_4 emissions from cattle are greatly needed. Furthermore, enteric CH_4 represents an energy loss to the animal (2-12% of gross energy intake; Johnson and Johnson, 1995). Measures of Enteric CH4 and carbon dioxide (CO_2) emissions and oxygen (O_2) consumption can be used to estimate energy expenditure of cattle (Brouwer, 1965;

Kaufmann et al., 2011), thereby providing a means to investigate ways to improve the energetic efficiency of beef cattle. Accordingly, methods to measure gas exchange of ruminants are important for the long term economic and environmental sustainability of ruminant livestock production.

There are several options available for researchers to measure gas exchange of cattle. Respiratory chambers have long been used to conduct indirect calorimetry for energetic research (Kleiber, 1935). These systems place an animal inside enclosed chambers that allow frequent measurement of gas exchange from the cattle. Increased scientific and societal interest in measuring and monitoring enteric CH_4 emissions has led to an increased number of experiments using respiration chambers in research. In an attempt to make respiration chambers cheaper, a head box system has been developed (Place et al., 2011). Head-boxes function similarly to whole body respiration chambers, but only contain the animal's head. Whole body respiration chambers and head boxes provide high resolution data and have long been considered the gold standard of gas exchange measurement systems (Hill et al., 2016). However, these systems are labor intensive, can only measure a small number of animals at a time, remove cattle from their management routine, and therefore may not represent expected production responses.

The sulfur hexafluoride technique (SF6; Johnson et al., 1994) was developed to quantify CH4 enteric from unrestrained cattle. This technique inserts a SF6 containing bolus with a known permeation rate into the rumen and then released gases are captured into a canister with a vacuum attached to a harness that is placed on the animal. The concentration of SF6 and CH4 within the cannister are analyzed and daily emissions of CH4 are then calculated based on the known quantity of SF6 released by the ruminal bolus. The SF6 technology is also labor intensive, making it difficult to measure a large number of animals at a time. The frequent handling of animals required outside typical management may impact the interpretation of results. Furthermore, the SF6 technology is integrative, making it impossible to assess diurnal variations in CH_4 emissions.

The large ruminant GreenFeed (C-Lock Inc., Rapid City, SD; Hristov et al., 2015; Gunter and Beck, 2018) is the most recent methodology to measure enteric CH4 emissions from 80 to 2000 g CH4 in beef cattle, and pubertal age to lactating dairy cattle. The recommendations listed are specific for the large ruminant GreenFeed system. Small ruminant GreenFeeds are recommended for beef and dairy animals under six months of age, or for research involving sheep and goats. This system is like a head box system, but estimates emissions based on multiple spot samples collected while the animal is visiting the GreenFeed unit. The GreenFeed unit allows for many unrestrained animals to be sampled at a time, 20-50 animals per GreenFeed and requires less labor and animal handling than the SF6 technique. In tie stall or individual pen settings, spot sampling methodology requires manual labor to move machines between animals resulting in 25 animals or fewer per machine to reach maximum of 3.5 hours per sampling session.

Global use of the GreenFeed technology has increased rapidly (Figure 1) since introduction in the mid-2010s with over 700 units distributed and in use (C-Lock product log tracking process). Accordingly, when the GreenFeed number is plotted against date received, an exponential increase in adoption can be seen (Figure 1). The widespread adoption of the GreenFeed technology necessitates a standardized procedure for experiments to be repeatable across different laboratory groups. Our last review, with recommendations on using the GreenFeed was written approximately 7 years ago (Gunter and Beck, 2018). Many recommendations have remained the same; however, key method development experiments have been conducted since. Accordingly, the objective of this manuscript is to provide an updated recommendation for using the GreenFeed system across contrasting experimental settings, livestock type (beef and dairy), and management settings (pasture and grazing). We ultimately hope to provide guidance on implementing the GreenFeed system and to standardize procedures.

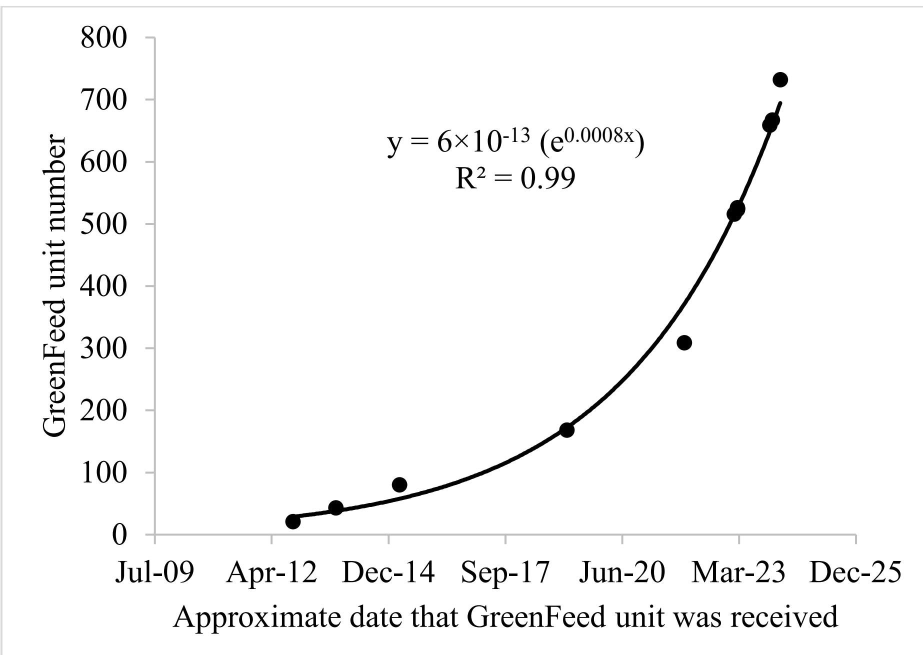


Figure 1. *GreenFeed unit number (13 units ranging from #21 through 732) against date received, demonstrating that use of the GreenFeed system has exponentially increased.*

The GreenFeed System

The GreenFeed system (C-Lock inc., Rapid City, SD) is a modified open-circuit respiration chamber, functioning similar to a head box chamber (Place et al., 2011). However, rather than restraining the animal in a metabolism stall and continuously measuring gas exchange, estimates of daily gas exchange are the average of multiple spot samples. Several studies have compared the GreenFeed approach, describing the pros and cons relative to other *in vivo* systems for measurement of gas exchange of cattle (Hammond et al., 2015; Huhtanen et al., 2015; Hill et al., 2016; Gunter and Beck, 2018; McGinn et al., 2021; Ma et al., 2024).

Figure 2 presents an overview of GreenFeed systems and the primary components and features of a unit. GreenFeed units are designed and manufactured in variable configurations (Panel A – Free stall unit #309; Panel B – Pasture unit #659; Panel C – Pasture Unit; Panel G – Tie-stall unit #527 with lactating cows; Panel H – Tiestall unit #323 with prepubertal heifers) to meet the use needs within the animal environment (inside, outside, open pen, pasture, freestall, tie-stall, etc.) and size/type of animal measured (young, mature, dairy, beef, etc.). C-Lock, Inc. uses sequential numbering to identify the order of production and to link the machine in the field with centrally held user interface connectivity including calibration and data accumulation components. Cell phone applications are used to connect in field controls with centrally controlled user interface information.

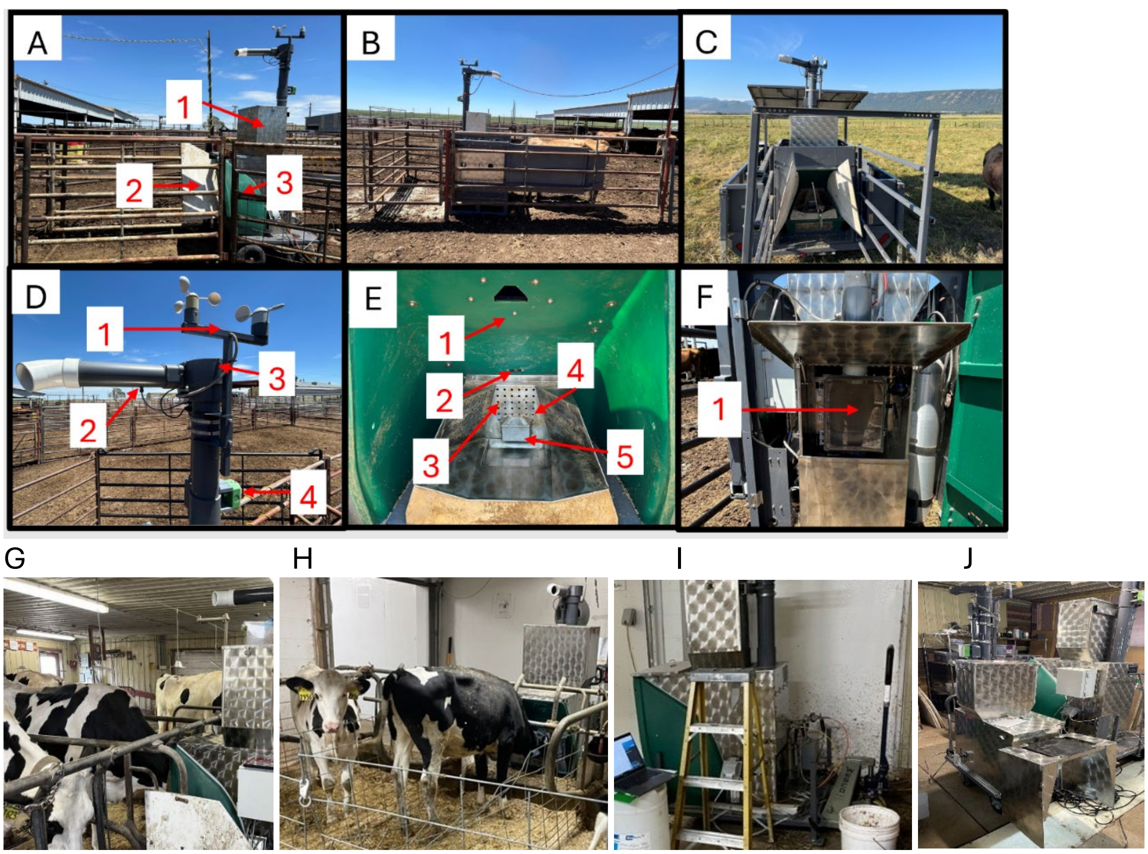


Figure 2. *Pictures of GreenFeed unit types and components of the system. Panel A: a free stall unit, item 1 is the feed hopper, 2 is the wooden wind block, 3 is the GreenFeed hood; Panel B: a skid unit; Panel C: a pasture unit; Panel D: item 1 is the weather station measuring wind speed and direction, 2 is the air subsample line, 3 is where the fan is housed, 4 is the airflow rate sensor; Panel E: inside the GreenFeed hood, item 1 shows where the EID reader is located, 2 is the head proximity sensor, 3 is the feed pan with air intake manifold, 4 shows where the CO2 release system is inserted for*

CO2 recoveries, 5 shows where feed is dispensed; Panel F: the back of the GreenFeed showing where the primary air filter is located.; Panel G: the GreenFeed in a tie-stall setting with short airstack adaptation and tie-stall cart; Panel H: the GreenFeed in a tie-stall setting with short airstack adaptation and tie-stall cart with 6-month old animals; Panel I: Tie-stall cart adaptation on large ruminant GreenFeed; Panel J: Small ruminant GreenFeed unit Figure 2, Panel D identifies the 'stack' of instrumentation used to collect environmental and emissions data. If using the system in a tie-stall setting, an adapted, shorter stack must be installed to accommodate the roof clearance (Figure 1, Panel H). Panel D, Item 1 – a weather station, accounting for wind speed and direction when GreenFeed units are used outdoors; Item 2 – air sample collection tube for subsequent analysis of gas concentrations; Item 3 – housing for the fan that draws air around the animal's head and through the system for gas collection; Item 4 - airflow sensor.

GreenFeed units require access to electrical power (110 V or solar panel with battery storage) with the choice based on where the unit will be placed and the intended use. Protection of the primary unit on all sides, except the location where animals enter and measurement data are collected, is essential. Experience indicates that free stall units require additional protection in the form of gating/fencing to prevent animal access, damage, and avoid multiple animals in the GreenFeed that could compromise gas sampling. Additional anchoring of GreenFeed units into concrete may be necessary to avoid movement of the unit by animals. Skid units and wheeled pasture units are typically built more rugged to withstand greater animal pressures. For research settings where GreenFeed units are placed in pens of cattle or in pasture settings, the recommendation is to invest in units built strong and designed to protect the primary unit. Compared to the carts used in a freestall or pen setting, tie-stall units need an adapted cart is used to house the GreenFeed unit (Figure 2, Panel I). The cart and lifting mechanism allow ease of turn the unit around turns and raising the unit to allow ease of use by the animals (Figure 2, Panel G).

GreenFeed units share similar components (Figure 2) including: Panel A, Item 1 - feed hopper storage for pelleted supplement; Item 2 - a wind block (free stall unit, wooden or plastic attachment) or integrated solid alley sides (skid or wheeled systems); Item 3 - the hood where cattle place their head for emission measurement. The wind block attempts to minimize the effects of wind and the influence of other animals while the cattle are visiting the GreenFeed. Figure 2, Panel E describes the inside the GreenFeed hood. Panel E, Item 1 – Radio-Frequency Identification (RFID) recorder; Item 2 – Animal head proximity sensor; Item 3 - feed pan with air intake manifold; Item 4 – location for the CO_2 release system is inserted for CO_2 recovery; Item 5 – location where feed is dispensed. Animals are trained to place their head into the GreenFeed hood using a feed supplement and access to the hood across time points is established based on the research protocol. Electronic Identification (EID) tags (placed in either ear) link to RFID to record entry and exit times for each animal for all visits. The proximity sensor measures the animal's head location while visiting the GreenFeed. Air is pulled through the feed pan, capturing the gaseous emissions as the animal consumes the feed, and further analyzing the air subsamples for specific gas concentrations.

Figure 2, Panel F shows the back of the GreenFeed, where the primary air filter can be accessed. Figure 3 panel A shows a dirty primary air filter (item 1) housed inside the plastic box at the back of the GreenFeed. Item 2 of panel A of Figure 3 shows where air flows from the GreenFeed hood and is drawn through the primary air filter, to initially remove dust and large particles from the air. In Figure 3, panel B the in-line sample filter, which provides another level of filtering following the subsampling of air that occurs at the top of the PVC stack.

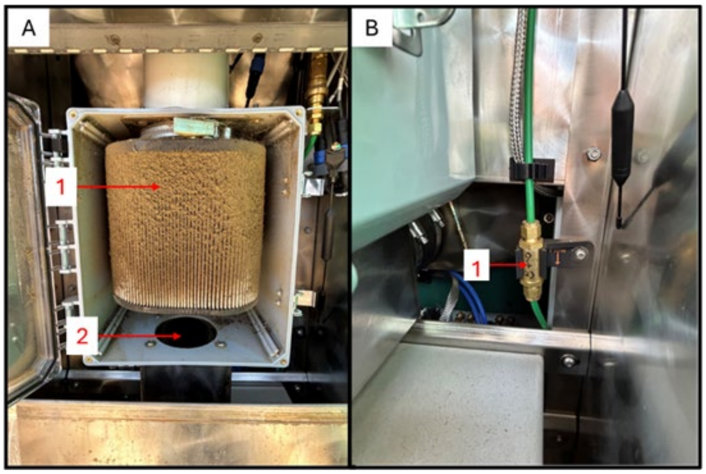
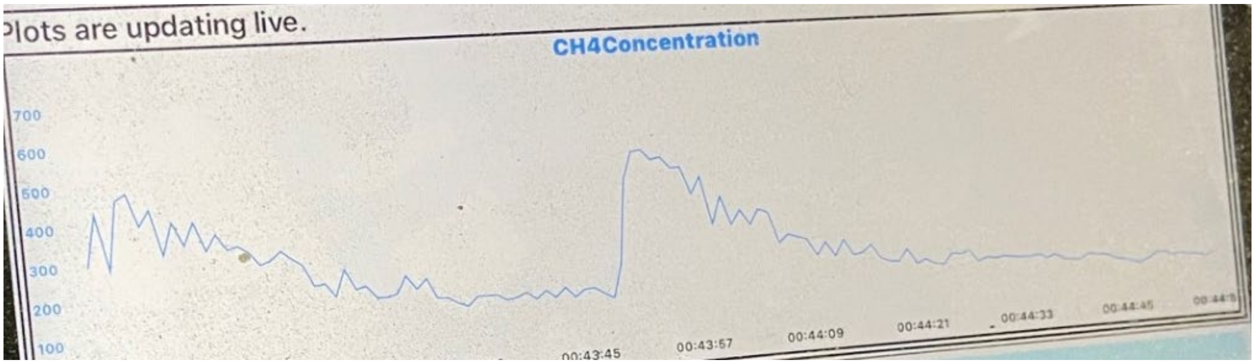


Figure 3. *The primary air filter and in-line sample filter.*

Figure 4 is a picture of the electronics box, which contains sample pumps, gas analyzers, and other electrical components for operating the GreenFeed. This box is located underneath the feed pan as shown in Figure 2, Panel E, Item 3. Item 1 of Figure 4 shows where subsampled air enters the electronics box. Item 2 of Figure 4 shows the sample pump to send subsampled air to the non-dispersive near-infrared CO_2 and CH_4 sensor (item 4) and item 3 shows the sample pump to send air to the paramagnetic O_2 sensor (item 5). Depending on the gas analyzers ordered, there may be more sample pumps and gas analyzers. Item 6 shows where air exits the electronics box after being analyzed by the gas analyzers. Item 7 is the modem, which connects the GreenFeed unit to WIFI. The researchers order a SIM card through a mobile phone company and inserts this card into the modem. We recommend unscrewing the modem from the unit so that you can more easily access the side of the modem to insert the SIM card. Item 8 is a beagle bone, which provides Bluetooth capabilities for the GreenFeed, so that the system can be operated through the “Control Feed” cell-phone app. It is recommended having designated tablets per unit when spot sampling in tie-stall to use the “Control Feed” app. This aids in monitoring each machine for proper head proximity and monitoring raw CH_4 emissions to aim for a minimum of three eructation sessions per sample collection time point (eructations in lactating tie-stall settings have been determined to average between 1 to 1.5 minutes per eructation bouts).



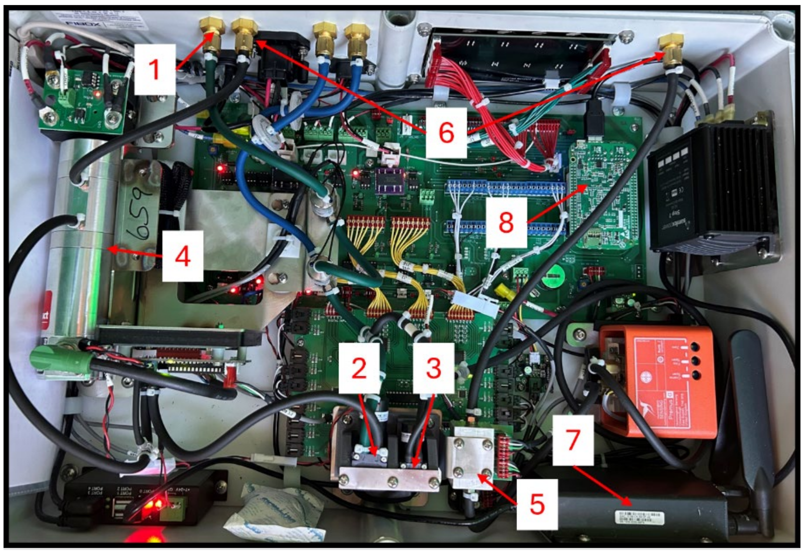


Figure 4. *The electronics box which contains sample pumps, gas analyzers, and other electronic components. Item 1 shows where subsampled air enters the electronics box. Item 2 is the sample pump for the carbon dioxide (CO2) and methane (CH4) analyzer. Item 3 is the sample pump for the oxygen (O2) analyzer. Item 4 is the non-dispersive near-infrared CO2 and CH4 analyzer. Item 5 is the paramagnetic O2 analyzer. Item 6 shows where the gas flows from the sensor out of the electronics box. Item 7 is the cellular modem. Item 8 is the "beagle bone" providing Bluetooth capabilities.*

Recommended Protocol

The following sections outline GreenFeed use recommendations based on manufacturer, experiential, and experimental evidence from previous research by the author group and others in the research community. The processes, practices, and procedures provided offer a starting point and a base level of background for research conducted using GreenFeed systems. They may not be applicable in every experimental setting, nor should they be considered the only approach to achieving research goals.

General Maintenance**:** Guidance for the general maintenance of the GreenFeed units can be found online (URL: https://docs.c-lockinc.com, accessed 06/27/2024).

- ***Air filters:***

  Air flow rate is a critical component of effective gaseous emissions estimation. Airflow through the feed pan collects dander from the animal and dust from the environment and feed pellets offered. The primary air filter (Figure 2, Panel F) is located in the back of the unit. Accumulation of dirt (Figure 3, Panel A, Item 1) can significantly reduce airflow. Manufacturer recommendations indicate that the primary air filter be replaced when airflow drops below 26 L/s in large ruminant GreenFeeds and 10 L/s in small ruminant GreenFeeds. Experimental support for this threshold is discussed in greater detail below. When replacing the primary air filter, we recommend scooping out any dust buildup under the filter and vacuuming the box completely after each experiment. The in-line air sample filter (Figure 3, Panel B) is recommended to be replaced at least once a year. Of note, the in-line filter is directional, and installers should ensure that the arrow on the filter points in the direction of airflow (down and toward the electronic box).

- ***Calibration and CO_2 recovery***

  We recommend contacting C-Lock Inc. Customer Support for specific instructions and guidance for calibration of gas concentration sensors on each GreenFeed unit. Proper and timely calibration and re-calibration of sensors ensure accurate results. C-Lock units under 300 have autocalibration where 0 and span gas tanks. Newer GreenFeed units are commonly equipped with auto-recovery systems that only include a zero-gas tank, by which C-Lock Customer Support can establish timelines, as frequently as daily, for remote recalibration. Both systems serve the same purpose of gas recoveries that recalibrate the sensors. We recommend calibration after primary air filter replacement and at a minimum weekly while in operation. Manual calibrations using filled foil gas sample bags can be conducted. Auto-calibrations can be initiated in the “Control Feed” cell-phone app.

  The standard calibration procedure initially releases a zero gas, which contains 20% O_2 with nitrogen to balance, but no CO_2 or CH_4. Following the release of the zero gas, a span gas (i.e., calibration gas with known concentrations of gases) is released to the gas concentration sensors. The difference between analyzed concentration of the span gas is compared to the sensor measured concentration of these gases to generate a correction factor. These should be checked against previous standard calibrations to ensure that the correction factors are not changing to a large degree, 10-20% change. If a significant change in correction factor does occur, it is recommended for C-Lock Inc.’s support to check each standard calibration to ensure that everything is functioning appropriately.

Carbon dioxide recovery rates are used to assess the gaseous capture rate of the machine and can be used as an indirect validation of sensor calibrations. Carbon dioxide recoveries can be determined less frequently than standard calibrations. We recommend completing CO_2 recovery rate calibration at least monthly, after changing the primary air filter and conducting standard calibration processes. Carbon dioxide recoveries use a CO_2 release system (Figure 5, item 1), with a 90-g CO_2 canister (e.g., JT 90-g prefilled paintball CO_2 tank; Montreal, Canada) attached (Figure 5, item 2). The steps used for CO_2 recoveries are:

- The CO_2 release system and the attached CO_2 cylinder should be weighed for an initial weight and the start time should be recorded. Note: The largest potential for error during this process is in weighing the CO_2 release system at the start and end of each CO_2 release event. This should be done with at least to the nearest 0.1-g. At this level of resolution, any amount of wind can make the scale fluctuate and make obtaining an accurate weight difficult. It is necessary to use some means to collect these weights in the absence of wind. This could mean weighing indoors or using some type of box where the scale is housed, and the CO_2 recovery system can be weighed within. Accumulation of ice on the cannister is also a very common cause of error. Wiping CO_2 canisters with ethanol aids in rapidly melting the ice and allow for timely weighing of the canister. inserted into the air-intake manifold of the feed pan, where a washer has been welded as shown in Figure 2 panel E, item 4. The CO_2 release system valve is then turned to release CO_2 for approximately 3 minutes. In each sequential CO_2 release, increase the time by 2 minutes (3, 5, and 7 minutes per release) to ensure 20 g release per minute. When the temperature decreases below 4°C, consider increasing time increase per release by 3 minutes (3, 6, and 9 minutes per release).
- After these 3 minutes the system is weighed, and the amount of CO_2 released can be calculated by the difference between initial and final weight of the CO_2 release system plus the CO_2 cannister.
- This procedure is repeated at least 4 times, with two minutes separating each release step.
- Login to your GreenFeed account, select the data tab, as depicted in Figure 6 item 1, then select the CO_2 recovery test tab as shown as item 2.
- From the available list of systems (Figure 6 item 3), select the appropriate GreenFeed unit.
- Enter the correct start date and time and an appropriate duration (give yourself enough room on either side of the recovery) and select "Show Recovery (Figure 6 item 4). The graph should display the CO_2 recovery event with clear peaks where the CO_2 was released.
- Select "manually select all start/stop times sequentially" (Figure 6, item 5) and an ellipsis (i.e., ...) should appear in the start time column for the first recovery event. Manually click on the figure the beginning and then end of

each CO_2 release peak (Figure 6, item 6) and the start and stop times will automatically appear in the cells.

- Enter the initial and final weight of the CO_2 release system plus CO_2 cylinder (Figure 6, item 7) and then select "Calculate Masses" (Figure 6, item 8).
- The "% recovery" column should automatically fill (Figure 6, item 9) and you should check that the average of these values are close to 100%, (100% ± 5%). Note: it is good practice to ask C-Lock inc. for support to check these CO_2 recoveries. Recoveries may decrease by 10% when temperatures drop below 4°C.

To facilitate automatic calibration and CO_2 recovery processes, new GreenFeed units can have a "gas mass flow controller" installed which can accurately release gases from cylinders with known gas concentrations. This new approach is undergoing validation to replace manual CO_2 recovery; however, currently the recommendation is to maintain manual processes until validation is complete.

Figure 5. *The CO_2 release system for CO_2 recoveries*

Figure 6. *Web-interface for determining carbon dioxide recovery rates. Items 1 and 2 show the tabs to select to reach the carbon dioxide (CO_2) recovery web interface. Item 3 shows where to select the appropriate GreenFeed unit. Item 4 shows where to initially identify the recovery by entering the start time and duration. Item 5 shows where to select so that start and end time of each CO_2 release can be selected on the graph. Item 6 shows an example of where you would click on the graph to identify the start and end time of each CO_2 release. Item 7 shows the columns to enter the weights measured in the field. Selecting the button identified by item 8 tells the unit to calculate GreenFeed estimated mass of CO_2 released. Item 9 shows the calculated CO_2 recovery, comparing the mass of CO_2 release determined by manually weighing with the GreenFeed estimated CO_2 release.*

Animal, Feed Supplement, and GreenFeed Setting Recommendations:

Training to the GreenFeed: Regular visits to the GreenFeed unit are essential to gathering enough observations per animal and per treatment in experimental settings. Our experiences indicate that training animals in drylot pens, where animal proximity to the unit are controlled, help establish regular visits to the measurement hood. In an initial research study (Beck et al. 2018) conducted in a 40 ha, tall-grass prairie research pasture, researchers reported that of 25 candidate cattle, only 18 cattle used the GreenFeed and that only 14 of 18 cattle had adequate visits for use in CH_4 analysis (only 56% of the 25 cattle exposed to the GreenFeed). In subsequent experiments (Beck et al., 2019; Thompson et al., 2019; Beck et al., 2021; Beck et al., 2022; Beck et al., 2023; Proctor et al., 2024) cattle were trained to the GreenFeed unit in a dry-lot pen and a high proportion (~80%) of available cattle provided adequate visits to be included in research trials.

Our experiences indicate that cattle are initially more resistant to visit the GreenFeed unit if they must enter an alley and/or when wind blocks near the hood are present. Where feasible, we recommend removal of wind blocks in early training processes. In newer GreenFeed Units (Figure 7, Panel D – pasture unit; Panel E – skid unit) the alley may be integrated into the frame, making wind block and or alley removal unfeasible. In these situations, we recommend initially opening the alley to its widest setting and narrowing the alley over time to reduce risk of multiple animals attempting to enter and interfere with access. Additionally, in certain grazing situations using the pasture trailers, it may also be recommended to leave the alley in the up position, leaving the greenfeed entirely open, to get animals accustomed to use the units. The visit numbers can be monitored, and once the animals have learned to use the equipment, the alley can be lowered.

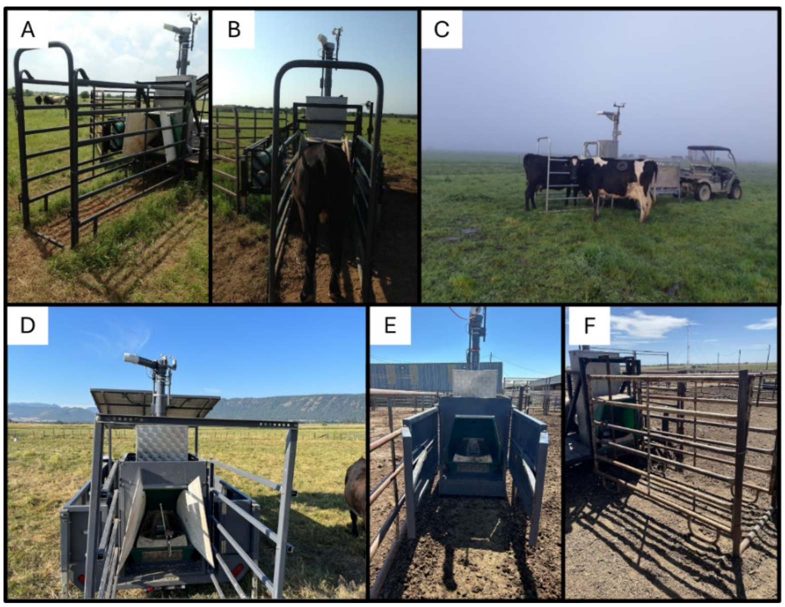


Figure 7. *Different GreenFeed units and alley way set-ups that have been used.* ***Panels A, B****, and* ***C*** *show different alley set ups for two different pasture units.* ***Panel D*** *shows the alley set up, which is integrated into a newer pasture unit.* ***Panel E*** *shows the integrated alley for a skid unit.* ***Panel F*** *shows an example alley set up for a free stall unit.*

During early training, we recommend adding the pelleted supplement or some other attractant into the feed pan a few times a day to stimulate interest. Ground feeds, including distillers dried grains with solubles, soybean meal, or ground corn in minimal (<100 g amounts) can be used as attractants during training and placed by hand into the feed pan. However, they are not recommended for use in the feed bin during sample collection. Further discussion on this topic is below.

Following exploration and awareness, we recommend programming the GreenFeed unit to dispense feed every 24s while the animal's head is identified as properly positioned in the hood, with a minimum time between visits where feed is delivered of 4 hours in a feedlot or pasture setting. After approximately one week, panels used to form the alley can be added, the width of the stationary alley adjusted, and wind blocks reattached to meet experimental settings. Flexibility in decisions regarding training timelines is essential, as each group of cattle is different in their rearing background, stage of life, and temperament and environmental conditions including season and weather events can require training adjustments. Experience indicates that 2-8 weeks of training may be necessary, and we recommend planning for a 4-week training period in most scenarios.

Studies (Gunter and Beck, 2018; Beck et al. 2018) report that 20 to 30% of cattle may not adequately train to the GreenFeed unit; therefore, researchers should plan to train more animals than required for a given experiment. Identification and selection of individual animals for experimental use can be determined by data generated from visits to the GreenFeed unit during training. Where feasible and the risk of creating treatment bias is low, individual visit data may be used to more uniformly allocate animals to experimental treatments based on similarity in GreenFeed visit criteria. Beck et al. (2018), using random allocation from a trained population, reported only one animal visited the GreenFeed in the control treatment group.

In a tie-stall setting where spot sampling is used, Hristov et al. (2015) has detailed adaptation tothe GreenFeed unit and training animals to the machine. Recently (Montes et al., unpublished), in studies conducted at the USDA Dairy Forage Research Center with 60 or more midlactation Holstein cows, after initial placement of bait feed on animal's typical ration and feed manger, 3 separate daily training sessions of 2 - 5 minutes each trained over 97% of animals. Occasionally, feed drops are set at 30 s during initial training and increased to 45 s during collection to minimize GreenFeed supplement intake. When dairy heifers 6 months of age were introduced to the GreenFeed, the GreenFeed was placed in within view of the animals for at least 2 days prior to exposure, with the chime and feed drop activated at least twice per day during that time. If able, consider providing the grower typically fed to the animals as GreenFeed bait to encourage approaches (Wu et al., 2024). When comparing emissions between groups of lactating animals previously trained to a GreenFeed system versus those that had not been trained. Both groups were retrained to the GreenFeed in a freestall setting. No difference occurred in visits to the GreenFeed or average daily emissions (FitzGerald and French, 2024). Results indicate blocking for previous training is not required Introductions of novel procedures to dairy cattle previously demonstrated altered animal behaviors, questioning measurement reliability (Hemsworth et al., 1996).

Number of Animals per GreenFeed: The number of animals per GreenFeed unit is dependent on their proximity to the system and animal stocking density. In pasture experiments, 20 to 25 animals per greenfeed is recommended, 20 may be the optimal number in extensive pastures with low stocking density, and up to 25 in small pastures with greater stocking density. In confined settings (Beck et al., 2023;

Proctor et al., 2024), reports of success with 27 animals per GreenFeed unit are demonstrated, with a note that traditional feed bunk space limited their opportunity to evaluate greater animal numbers. Other researchers have successfully evaluated up 50 animals per GreenFeed unit in a confined setting (Sara Place, Colorado State University, personal communication). Published and anecdotal information support allocation of 40 to 50 animals per GreenFeed unit in confined experimental settings, adjusted based on training success and cattle temperament within the context of experimental conditions. In a tie-stall setting, animal numbers are dependent on management routines (e.g. milking and feeding times). Therefore, 20 to 25 animals allowed for up to 3.5 hours of spot sampling assuming 5 minutes GreenFeed sampling per cow and 2 minutes rest period between each animal. Feed in manger should be removed prior to placing unit in front of the animal.

Considerations for Feed Supplement: GreenFeed systems offer pelleted or texturized supplements based on researchers choice and nutrient contraints. The feed is dispensed in measured increments via a cup embedded on a rotating cylinder (Figure 8), to attract the cattle to visit the emissions collection hood. The supplement is designed to attract cattle to visit the GreenFeed, offering composition and palatability attributes, and commonly supplemental nutrients, that differ from the base diet available in the experimental setting. Examples of successful pelleted supplements and their use scenarios from GreenFeed experiments include: a) 97% soybean meal and 3% molasses pellet used in tall grass prairie pastures throughout the summer; b) alfalfa pellets offered to cattle grazing in mixed grass prairie pastures and in ; c) wheat middlings offered to cattle grazing winter wheat; d) a multi-feedstuff commercial alpaca and llama pellet offered to dairy cows fed with access to alfalfa baleage and grazing perennial ryegrass pastures (Pelleting and consistent texturized feeds are used to reduce dust, allowing the unit to maintain airflow above 26 L/s, as previously discussed. Feeding supplements with greater levels of fines/dust particles will require more frequent air filter cleaning/replacement and decrease consistency in the mass delivered at each rotation. Experience indicates that pellet quality and the number of fines is influenced by formulation and product handling. For pelleted supplements with excess starch, fat, too little heat during the pelleting process, fines can increase in the pellet. If there are excess fines/dust, pouring the feed from the original container in the presence of natural or artificially generated wind can reduce dust levels in pellets used in the GreenFeed hopper. Moisture can also impact pellet integrity after delivery and form larger clumps of feed that can jam and impact equipment from working properly.

Figure 8. *The cylinder and cup responsible for dispensing the pelleted bait supplement.*

The weight of feed supplement offered at each visit is important for estimation of dry matter intake (DMI) consumed per visit and summed across a defined time frame for each animal. The user establishes the number of daily drops per animal for each day of the experiment, estimating DMI from an estimate of average drop weight multiplied by the number of experimental days. The cup dispensing/dropping pellets is a fixed dimension; therefore, pellet diameter influences weight of each drop from the cup. To determine the average and standard deviation of individual drop/cup weight, we recommend weighing 20 individual drops. Using this technique, experience tells us that smaller diameter pellets appear to fill the cup more fully and consistently. For example, the average drop weight of a 4.8-mm diameter pellet (avg. 32.48 g, std. dev. 1.6 g, CV 4.9%) was heavier and less variable when compared with a 6.4-mm diameter pellet (avg. 30.08 g, std dev. 3.0 g, CV 10.0%). Knowing the impact of pellet diameter on weight and weight variation is important for two reasons. First, researchers can have increased confidence in pellet DMI from the GreenFeed when a smaller diameter pellet is used. Second, if researchers aim to use the pellet to dose an animal, such as adding an external marker (e.g. titanium dioxide, etc.) to measure fecal output (Beck et al., 2021), then a smaller variation in drop size may reduce variability in the average dose rate. An important assumption with analyzing feed drops from GreenFeed systems is that all feed dropped to an animal is consumed. Refusals recorded at the end of spot sampling prepubertal heifers noted a range in 5 to 75 g refusals of GreenFeed based on an average of 7 drops, 45 second apart (Wu et al., 2024).

Dry matter intake from the GreenFeed pellets can represent a relatively large percent of total DMI. For example, GreenFeed pellet intake represented 2.1% to 4.5% of total DMI in cattle (initial body weight = 279 ± 8 kg) grazing tall grass prairie pastures in late summer. In finishing beef cattle, pellet intake from the GreenFeed units represented 4.0% to 6.4% of total DMI (Beck et al., 2023; Proctor et al., 2024).

Prepartum dairy cows limit fed alfalfa silage had 2.6% to 4.7% of total DMI composed of GreenFeed pellets, whereas the same set of cows offered perennial ryegrass and white clover pastures had 1.7% to 2.7% of their DMI from the pellets (Beck et al., 2022). The contribution of GreenFeed feed supplement DMI to total DMI will be dependent on the researcher's GreenFeed feeding regime including number of drops per visit, the minimum time allowed between visits, the number of visits the cattle choose to make, and the DMI of the basal diet. Data on total drops per animal can be downloaded from the 'Animals' tab by selecting the 'Animal Statistics', selecting the GreenFeed unit of choice, date range of interest, and selecting the 'Download' function.

Researchers must consider the nutritive characteristics of the feed supplement and how they may impact the larger experimental objectives. As described in the previous paragraph, DMI from GreenFeed feed supplement can represent an appreciable amount of total DMI. Accordingly, if the aim of an experiment is to set/establish target dietary formulations/requirements, knowledge of the pellet intake's contribution of a particular nutrient is essential. For example, Proctor (2023) conducted an experiment to determine how feeding level of ruminal degradable protein (RDP) influenced nitrogen excretion and enteric CH_4 emissions. The treatments were formulated to provide 87%, 100%, and 113%, of total digestible nutrient (TDN) allowable microbial crude protein (MCP) synthesis (i.e., RDP required, NASEM, 2016), resulting in a low RDP, neutral RDP, and high RDP treatment, respectively. Based on the total DMI (base diet + pellet supplement), the *in situ* analyzed supply of RDP resulted in an RDP balance of 104.3%, 111.2%, and 122.2% of TDN allowable MCP synthesis. In contrast, without accounting for the GreenFeed pellet intake, RDP balance was calculated as 98.3%, 111.8% and 119.8% for the low, neutral, and high RDP treatments, respectively, values closer to the target levels (Proctor, 2023). Accordingly, the alfalfa pellets consumed through the GreenFeed provided 12.0%, 8.8%, and 9.1% of RDP intake, despite only representing 6.4%, 5.4%, and 6.0% of DMI for the low, neutral and high RDP treatments, respectively. In retrospect, the authors acknowledge the need to have identified a pellet supplement with a lower RDP content.

In grazing scenarios, the choice of feed supplement is also a critical component that must be considered while designing experiments. As previously mentioned, feed consumed from GreenFeed can often represent large proportions of the animal's daily intake. The choice of pellet attractant can induce unwanted supplementation effects, introducing confounding errors into a given experiment (Moore et al., 1981). Issues arise when the GreenFeed supplements are in contrast to the pasture nutritive value, altering the consumption of forage on pasture through additive, substitutive, or combined effects (Moore et al., 1981). Whenever possible, it is suggested that the pellet attractant used be as similar in nutrient composition to the pasture where the cattle are grazing. For instance, Mombach et al. (2018) pelletized Tifton 85 Bermudagrass hay, which was similar in nutrient composition to the *Brachiaria brizantha* pastures where the steers were grazing. By adding vanilla extract to the pelletized hay, the steers were successfully trained to the GreenFeed equipment, eliminating the need to use other supplements (such as protein supplements) as an attractant, which were in direct contrast to the composition of the pastures.

In randomized complete block designs, Latin Squares or other, feed can be included in the common total mixed ration during times where GreenFeed samples are not being collected. During sampling periods, the feed that would normally be provided through the total mixed ration diet (TMR) can be removed from the mix and instead fed through the GreenFeed. Assuming cows consume all the feed from the GreenFeed, this will not hypothetically impact the intake of the TMR. Similarly, when conducting experiments using GreenFeeds in automatic milking systems (AMS), using the same pellet fed through the AMS in the GreenFeed allows adjustments to the total pellet through the AMS to be adjusted so the contribution from the GreenFeed supplement does not alter the contribution of concentrate to forage in the overall DMI. For example, if targeting 1 kg intake through the GreenFeed, remove the same amount at each milk volume level in the AMS feed allotment.

Other Considerations: Securing the GreenFeed units for their use is vital to the structural integrity of the equipment, but also to the quality of the data collected. Each unit contains critical components that must be protected from damage by cattle. Depending on the type of unit, special considerations must be considered for protecting the equipment. Newer model pasture trailers have been improved where most sections are block off by metal panels and parts are well out of reach of most grazing animals. However, it is recommended that special precautions be taken to protect the front side of the trailer, opposite to the alley. There is not a one-size-fits all manner of achieving this, as it all depends on the conditions for each situation. In improved pastures, where mobile electric fencing is available a fence wire can be run across the length of the GreenFeed trailer to block and protect off the entire side of the machine. Since the fence wire is electrified, a polyvinyl chloride (PVC) pipe can be cut and adjusted to fit on the side of the metal trailer to be used as an insulator, preventing the flow of electricity. This practice is beneficial for situations where the units require frequent movements. In other situations where electric mobile fencing is unavailable, corral fence panels may be set up to create a perimeter around the GreenFeed.

User Defined Visit Settings: The goal is to have cattle remain with their head in the hood of the GreenFeed unit for ≥3 minutes per visit and to have visits spread as evenly as possible throughout the day. To achieve these goals, the researcher can program the number of drops per visit, the number of seconds between drops, a minimum time between visits, and the maximum number of visits per day for an individual animal's GreenFeed visit. We typically utilize 24 seconds between each drop with 8 drops per visit (Beck et al., 2021; Beck et al., 2022; Beck et al., 2023; Proctor et al., 2024) or 30 seconds between each drop with 6 drops per visit (Beck et al., 2018; Beck et al., 2019; Thompson et al., 2019). For research studies more concerned with the proportion of DMI and associated nutrients from consumption of pellets offered in the GreenFeed unit, establishing a 30 second interval and 6 drops per visit will, in theory, reduce pellet DMI by 25% relative to a 24 second interval with 8 drops per visit. Importantly, Gunter and Bradford (2017) did not determine an effect of pellet dispense time interval on estimates of CH_4 or CO_2 emissions and concluded that as long as the cattle remained for more than 3-minutes per visit, the number of drops and the time interval between drops did not matter. In tie-stall settings, or when spot sampling is used, 5 to 6 drops with 45 seconds between each drop can be used (Nelson et al., 2024). In tie-stall studies with dairy heifers 6 months of age to 18 months of age, settings should be adjusted changed to 8 drops with 45 seconds between drops to target 5 to 10 minutes of sampling data (Wu et al., 2024). Due to the head proximity of younger animals, more time in the GreenFeed is required to meet the threshold for good data during processing. Also, head proximity settings may need to be decreased to 600 to allow for proper dispensing of feed.

Programming to establish the minimum amount of time between visits and the maximum number of allowable visits per day allow researchers to spread visits more effectively across the day and help account for diurnal variation in gas exchange (described in greater detail below). As the number of allowable visits per day is increased, DMI from supplemental pellets will likely increase; therefore, in high DMI animal experiments (lactating dairy cows, mid- to late-finisher cattle) we suggest increasing the number of allowable visits and decreasing the minimum time between visits For example, a dairy cow with an expected DMI of the basal TMR of 25-kg per day, could receive 0.5-kg of GreenFeed pellet per day and this would only represent 2% of their total DMI, whereas a grazing steer with a daily DMI of 6-kg per d of the basal forage consuming the same amount of GreenFeed pellets per day would have 7.7% of their total daily DMI from the pellets. Realizing that the more allowable visits per day the greater potential DMI from GreenFeed pellets exists, it may be logical to have a greater allowable daily visits and less minimum time between visits for experiments with cattle that have high DMI of the basal ration. This strategy would allow the opportunity for more visits per day in some experimental settings, without greatly increasing the proportion of pellet intake to total DMI. In a freestall dairy experiment, we allowed 8 visits per day with a minimum of 3 hours between visits, where DMI was expected to be 14 kg of alfalfa baleage per day during the dry period and then 18 kg per day of a perennial ryegrass pasture during the lactation phase (Beck et al., 2022). However, in this experiment, there was still only 2 visits per cow per day in the prepartum period and 0.9 visits per head per day in the post-partum period (Beck et al., 2022), indicating that perhaps the allowing 8 visits per day did not necessarily translate to greater GreenFeed use. Other freestall research on late lactation Holsteins demonstrated allowing 6 visits per day or minimum of 4 hours between GreenFeed visits also resulted in an average of 2 visits per day (FitzGerald and French, 2024). In tie-stall facilities with dairy cows, allowing 6 visits per day compliment most management situations and spot sampling schedules. Another alternative to minimize intake at the GreenFeed, extending time between feed drops to 45 seconds and 5 drops per visit. This allows feeding into 4 minutes per session to meet the 3-minute minimum.

In other experiments with growing stocker cattle grazing warm season perennial pastures, cool season annual pastures, or fed finishing diets we allowed 4 visits per day with 4 hours of minimum duration between visits (Beck et al., 2019; Thompson et al., 2019; Beck et al., 2021; Beck et al., 2023; Proctor et al., 2024). In Beck et al. (2018) we programmed the GreenFeed to allow for 3 visits per day with 6 hours of minimum time between visits. This experiment had the worst number of useable visits per animal (and in total) with several animals not meeting the assigned threshold number of “good” visits per animal (i.e., 30 visits; Arthur et al., 2017). However, it is difficult to say if these programmed settings were the cause of this poorer visits or if it was all attributable to poor training. Regardless, we recommend programming the GreenFeed to allow for 4 visits per day with 4 hours of minimum duration between visits, as we have had success with this program previously. If your experimental settings utilize cattle with greater DMI of the basal diet, then setting greater allowable daily visits with smaller amounts of time between each visit may be appropriate, as intake from the GreenFeed pellets would represent a relatively small proportion of total DMI.

<u>Data Preprocessing</u>

When one downloads the processed data from the C-Lock inc. website, one obtains a row of data for each individual visit to the GreenFeed unit (further described in the subsequent section). From this, researchers must preprocess the data to arrive at a single estimate of gas exchange for each animal on trial. A researcher could use the dataset with each individual GreenFeed visit directly to explore treatment effects using a mixed model, and some laboratory groups do use this approach (Waghorn et al., 2016; Jonker et al., 2017). However, a single estimate, averaged over a defined time period, for each animal is necessary for subsequent calculations, such as CH_4 yield (g CH_4 per unit of DMI) and intensity (g CH_4 per unit of production). These data are used as input variables for equations, such as heat production (Brouwer, 1965; Kaufmann et al., 2011); to determine statistical associations, such as correlations between CH4 and DMI; or to identify low and or high emitting animals, such as residual CH4 production (Smith et al., 2021) – as a few examples. The following sections will describe common methods for the use and conversion of raw data collected as individual rows for individual animal GreenFeed visits to an estimate of gaseous emissions for an individual animal and recommendations for data preprocessing

Accessing Data and Important Columns: Data from each GreenFeed visit is available to download from the C-Lock Inc. website (URL: https://ext.c-lockinc.com/home) once logged into your account. After logging in, select the "Data" tab and then the "Processed Data And Support Files" tab. There will be a list of excel files that can be downloaded. This list will also show the file size and the last time that the file was modified. You can use the time that the file was last modified as an indication of which file contains the most up-to-date data. After downloading and opening the appropriate file, there will be a series of excel sheets. The sheet of primary interest is the "Events", which will have 25 columns. The column names may be different, depending on the GreenFeed unit. The most important columns include: the animal EID, the user defined animal visual ID, the GreenFeed unit number, the start and end times of the visit, the visit duration, the visit hour of the day (ranging from 0 to 23.99), and the average airflow (L/s).

Initial Data Cleaning: Initial data cleaning is based on a determination of adequate air flow for each visit logged with the removal of visits where inadequate airflow is reported. Adequate airflow is necessary to ensure total capture of the breath cloud generated as the animal's head remains in the GreenFeed hood. The experimentally determined threshold for adequate airflow is a minimum of 26 L/s. From 10-26 L/s there is a linear increase in estimated CO_2 and CH_4 emissions with increasing airflow and above 26 L/s there is a plateau, indicating complete capture of the cattle's emitted breath (Gunter et al., 2017). The research findings coincide with the recommendations of C-Lock Inc., and the recommendation is to remove any visits with average airflow rates below 26 L/s from subsequent analysis. For small ruminant GreenFeed units, data should be removed with airflows under 10 L/s.

The initial data cleaning protocol also establishes a time threshold for the minimum duration of a visit to the GreenFeed hood. C-Lock, Inc. GreenFeed software algorithms automatically remove any visit less than 2 minutes in duration from the reported data. To investigate the impact of a visit duration less than 2 minutes on CO_2 and CH_4 emissions, 24,195 visits recorded from four experiments (Beck et al., 2018; Beck et al., 2019; Thompson et al., 2019; Proctor et al., 2024) were obtained from C-Lock, Inc. Across the four experiments, 47.1% of observations were < 2 minutes in duration (Beck et al., 2024a). On average across the four experiments, a visit duration of <1 minute had 24.2% lower CO_2 emissions, 44.6% lower O_2 consumption, and 69.8% lower CH_4 emission estimates when compared with emissions estimates from visits ≥ 3 minutes in duration. When visits were ≥ 1 minute and < 2 minutes in duration, results indicated an 11.5% lower CO_2 emission, 12.5% lower O_2 consumption, and 22.9% lower CH_4 emission than those ≥3 minutes. Additionally, visits ≥ 2 minutes and < 3 minutes in duration had 7.3% lower CO_2 emissions, 5.4% lower O_2 consumption, and 4.9% lower CH_4 emission estimates when compared with estimates derived from visits ≥3 minutes in duration.

Using data from published reports (Beck et al., 2024a), analyses indicate that 40 visits of ≥2 minutes in duration per animal, or 30 visits of ≥ 3 minutes in duration per animal are required to provide adequate emission estimates, offering a 25% reduction in the number of required visits per animal if visit duration is lengthened to ≥ 3 minutes. This finding agrees closely with Arthur et al. (2017) who determined that for 2-minute visit duration threshold 45 GreenFeed visits are required and for a 3-minute visit duration threshold 30 GreenFeed visits per animal would be required. Dressler et al. (2023) also reported that for a 2-minute visit duration threshold a minimum 40 visits per animal were required; however, they did not assess visit requirements with a 3-minute visit duration threshold. Beck et al. (2024a) determined that for a 3-minute visit duration threshold achieving target visit number would require approximately 20 days in a grazing experiment and 13 days in a confined feeding experiment. Whereas, if a 2-minute visit duration threshold was used, approximately 25 days would be needed in a grazing study and 15 days in a confined experiment. Knowing that emission estimates are lower when visit duration is between 2 and 3 minutes, and the number of visits required per animal is greater for a minimum 2-minute duration, we recommend excluding all data for visits with less than 3-minute duration and including only data from animals with 30 or more visit records for further analysis. In tie-stall settings where animals are monitored throughout the data collection, 8 to 10 spot samples throughout are adequate for analysis. These sampling periods are spaced 5 – 7 hours apart based on milking times and management routines.

On Outliers: Outliers and extreme values can occur with all data, including gas exchange measured by the GreenFeed. The research community suggests removal of data point when it exceeds the variable's third quantile plus three times the interquartile range (Tedeschi, 2022). Beck et al. (2024b) summarized the number and (percentage) of outlier data points in five GreenFeed data sets. Using this threshold, there were 3 (0.2%) (Beck et al. 2018), 5 (0.4%) (Beck et al. 2019), 1 (0.1%) (Thompson et al. 2019), 0 (0.0%) (Beck et al. 2023) and 34 (0.4%) (Proctor et al. 2024) observations removed. Removal of the observations reduced estimated CH4 emissions by 0.2, 0.4, 0.3, 0.0, and 3.7 g/d for Beck et al. (2018), Beck et al. (2019), Thompson et al. (2019), Beck et al. (2023), and Proctor et al. (2024), respectively. Based on these five experiments where the number and percentage (≤ 0.4%) of total data points within an experiment were removed, removal of extreme values did not change the CH_4 estimate to a large degree. The frequency (number and percentage) of data points considered outliers should be investigated and consistently reported in findings. Where the number of outlier data points from GreenFeed recording are monitored and identified as being large or uncharacteristic, additional review of the recording and reporting process are warranted to identity sources of errors.

Methods for Arriving at a Single Animal Estimate: This section will describe arriving at a gas exchange estimate for each individual animal. These next approaches occur after you have removed visits with less than 26 L/s airflow rate and those that were less than 3 minutes in duration. Perhaps the most used method is simple arithmetic averaging. The arithmetic averaging approach simply averages across all GreenFeed visits within animal. There is some concern that this approach does not adequately account for diurnal variation, especially in instances where cattle preferentially visit the GreenFeed at a particular time of the day.

To better account for diurnal variation, Manafiazar et al. (2017) proposed time bin averaging. This method first averages visits into 6 (4-hour interval) to 8 (3 hour interval) time bins for each animal and then averages across the time bins within animal. This method weights each time bin equally, accounting for variation in the number of visits within a time bin. A potential issue with time bin averaging is that time bins with low visit numbers may be over-inflated (Beck et al., 2024a).

Recently, an alternative technique to account for diurnal variation when preprocessing GreenFeed data was proposed (Beck et al., 2024b). Their approach utilizes mixed model analyses to generate a least-squares means (LSMEANS) for each individual animal. The mixed model we used included each gas as the dependent variable, animal ID as the fixed effect, used airflow and visit duration as covariates (removed if they did not explain a significant amount of variation), and included date and hour of day by animal ID as random effects. The LSMEANS approach also provides a standard error of the mean for each animal's estimate. This affords researchers the opportunity to weight each animal's observations based on the standard error of the mean for that estimate as described by St-Pierre (2001). The researcher can determine if including this weighting procedure improves the model by reducing residual standard deviation (Beck et al., 2024b). Using the standard error of the mean to weight each animal's estimates resulted in lower residual standard deviation in 2 of the 5 experiments used for the investigation of Beck et al. (2024b).

There is a known diurnal variation in CH_4 emissions from ruminants across all production systems. However, the degree to which these emissions vary throughout the day appears to be diet and production system specific. Beck et al. (2024b) determined that cattle fed a finishing diet had a much larger diurnal variation in CH_4 emissions than grazing cattle. In pastoral systems, maximum CH_4 emissions appear to typically occur during the night (1800-0600 hours) and minimum CH_4 estimates occur sometime during the day (Gunter and Bradford, 2015; Beck et al., 2024b). However, this diurnal pattern is practically the opposite for cattle fed finishing diet, where maximum CH_4 emissions typically occur 2-3 hours after feeding and minimum CH_4 emissions typically occurred immediately prior to feeding (Beck et al., 2024b). In pasture cattle, the maximum CH_4 estimates were 21% to 45% greater than the minimum estimates, whereas the maximum estimates of CH_4 emissions were 77% to109% greater than minimum CH_4 estimates in cattle fed a finishing diet. In tie-stall and freestall settings for lactating dairy cattle, the diurnal pattern reflects initial feed delivery and the number of times the feed is pushed up throughout the day. The greatest increase is 1 – 4 hours following fresh feed delivery, with the lowest levels being within 3 hours of fresh feed delivery.

Time-bin averaging provided estimates of CH_4 that were only 3.9% greater than the arithmetic average estimates, on average for the pastoral based experiments used in the Beck et al. (2024b) investigation. Furthermore, the LSMEANS approach provided estimates of CH_4 emissions that were on average 0.1% greater than the arithmetic averaged estimates for those same pastoral based studies (Beck et al., 2024b). However, time-bin averaged and LSMEANS estimates were 8.7% and 7.2% lower, on average for the finishing studies (Beck et al., 2024b). These differences in average CH_4 estimates would indicate that accounting for diurnal variation is more important in finishing trials than in grazing studies. This is likely due to finishing trials having greater diurnal variation than grazing studies.

Based on our findings, time bin averaging increased the coefficient of variation by 9.1% relative to arithmetic averaging and 9.9% relative to the LSMEANS approach. This increase in unexplained error would require an additional 4-animal observations per treatment (an 22% increase) to detect a 10% treatment difference (assuming a beta of 0.20 and alpha of 0.05) compared with the LSMEANS approach (Beck et al., 2024b). Our experience indicates the LSMEANS approach accounts for diurnal variation with similar and lower residual standard deviation relative to arithmetic time bin averaging, respectively. We recommend researchers generate individual animal estimates using the described LSMEANS approach.

Research Gaps:

Additional opportunities exist to identify improvement methodology for utilization of GreenFeed for research purposes. In pasture and rangeland settings, attractants for co-locating with GreenFeeds to help with animals coming to GreenFeeds: water source, salt/mineral, supplemental tubs need to be evaluated. The influence of multiple water sources, shade and lighting throughout the day, forage quality, and impacts of larger extensive pastures on GreenFeed visits also needs to be elucidated.

Continued research is needed on emissions and their intensity throughout stage of life across ruminant species. Contributing to this variation is the impact on being moved between facilities (e.g. pasture to feedlot in beef; grower to heifer barns).

Conclusions

Throughout this protocol, we have provided recommendations based on experiential and experimental evidence. A summary of these recommendations can be seen in Box 1. For those approaches based on experiential evidence, we realize that others may utilize different approaches that are equally as good or better than those that we have recommended. If this is the case, we are not recommending that you change your existing protocols. However, for those protocols that are based on experimental evidence, we do recommend a change. These namely include data preprocessing methods. As the GreenFeed becomes more widely used there becomes a greater need for a standardized protocol so that experiments from various laboratory groups are repeatable and comparable to each other. The protocols listed herein represent our suggestion for such a standardized protocol. However, reviewers and editors need to ensure that researchers adequately describe the protocols utilized for their experiments, especially for data preprocessing techniques. As research on methane emissions continue, evaluating methane intensity in relation to production and product quality need to be considered on the whole-system food chain.

Box 1. Summary of the experiential and experimentally determined recommendations when using the GreenFeed system.

Experiential Recommendations:

- Training:
    - Dry-lot or freestall: Start with 20% more cattle than the study requires in a dry-lot pen. Allocate 4-weeks for training cattle to the GreenFeed. Begin with panels and wooden wind-blocks removed if feasible, or at least have the alley way as open as possible. As cattle progress in training add back elements slowly until the final set-up is in place.
    - Spot-sampling or tie-stall: Placing the unit in the animal housing area prior to training can help with adaptation. GreenFeed bait feed should be placed on the manger or on top of the animal's feed before approaching with unit for training. Schedule a minimum of 3 training sessions with unit of 5 minute each per cow prior to start of experiment.
- Animal Allocations per GreenFeed: We recommend 20-25 cattle per GreenFeed in pasture settings. More cattle may be used per GreenFeed in higher stocking densities and less in lower stocking density situations. In confinement studies, we feel comfortable with having 40-50 cattle per GreenFeed, if cattle are relatively calm and well trained. In tie-stall settings, total time for data collection is farm-dependent. For example, due to milking times, spot samples may only be collected over a 3-hour period, allowing for sampling on 20 – 25 cows (Assumes 5 minutes sample collection and 2 minutes rest between each cow).
- Bait Feed: use as small of feed as feasible as these will have more uniform drop weights. When choosing pellets or texturized feeds, you need to identify an option that will adequately attract cattle the GreenFeed, but consider how this feed will impact the larger objective of the experiment. Additionally, consistent bait feed nutrient and physical composition is crucial, as intake from GreenFeed can represent 1.5%-6.5% of total dry matter intake. You need to measure multiple individual drop weights to determine the average ± a standard deviation. We recommend collecting and weighing 20 drops. Considerations to the external total mixed ration, partial mixed ration, or routine supplement should be made to account for feed intake at the GreenFeed. For example, include 1.5% of dry matter as GreenFeed pellets in routine diet, then removing during sampling

times. If this is not possible, results and discussion of experiment should note ramifications on experimental objectives.

- User Defined Visit Settings: Set the GreenFeed to dispense 5-8 drops per visits, with 24-45 seconds between drops. Have 4-6 minimum time between allowable visits and 4 to 6 total allowable visits per day. If you are more concerned with how much GreenFeed pellets represent of total intake then set the system to allow 6 drops per visit with 30 seconds between drops and 4 allowable visits with 6 hours between visits. If you are less concerned with GreenFeed pellet intake then you can change all settings to more frequent times. The ultimate goal is to spread visits across the day and to keep animals in the GreenFeed for 3 minutes. In tie-stall settings, 5 – 6 drops per visit with 45 seconds between drops allows for 3 – 5 minutes of good data. Younger dairy animals 6 – 12 months of age may require up to 8 drops to allow to adequate time in the GreenFeed.

Experimental Determined Recommendations:

- Extreme data points do not significantly influence the overall estimates of gas exchange, based on our previously conducted experiments. If extreme data points do significantly influence your estimates, then there may be some larger issue occurring.
- With large ruminant units, remove visits where airflow rate is less than 26 L/s. With small ruminant units, less than 10 L/s. Below 26 L/s or 10 L/s on each respective unit causes incomplete gas capture to occur.
- Remove visits with <3 minutes in duration. Estimates from visits less than 3 minutes in duration are considerably lower than estimates ≥ 3 minutes in duration.
- Fit all visit data to a mixed effects model, where gas flux estimate is the dependent variable and animal ID is the fixed effect. Include date and hour of the day by animal ID as random effects. Generate least-squares means (LSMEANS) for each animal ID to provide that animal's estimated gas emission or consumption. Explore the appropriateness of weighting further statistical models using the standard error of the mean for the generated LSMEANS.

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