Measuring the respiratory gas exchange by grazing cattle using an automated, open-circuit gas quantification system[1]

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ABSTRACT: Ruminants are a source of enteric CH_4, which has been identified as an anthropogenic greenhouse gas that contributes to climate change. With interest in developing technologies to decrease enteric CH_4 emissions, systems are currently being developed to measure CH_4 emissions by cattle. An issue with grazing cattle is the ability to measure CH_4 emissions in open-air environments. A scientific instrument for this task is an automated, open-circuit gas quantification system (**GQS**; C-Lock, Inc., Rapid City, SD). The GQS is a head chamber that grazing cattle occasionally visit (3 to 8 min/visit; 3 to 6 visits/d), and while the animal consumes a small portion of bait (0.5 to 1.0 kg/visit), the GQS captures the animal's breath cloud by exhausting air through the GQS. The breath cloud is then analyzed for CH_4, CO_2, and O_2 concentrations. Data are hourly uploaded to a server where it is processed using algorithms to determine total daily fluxes. Several factors affect emission estimates generated by the GQS including the animal's visitation rate, length of sampling period, and airflow through the system. The location of the GQS is an important factor in determining the cattle's willingness to visit. Further, cattle need to be trained to use the GQS, which normally requires 4 to 8 wk. Several researchers have shown that 30 or more visits are required to obtain high-quality estimates of gas fluxes. Once cattle are trained to use the GQS, the bait delivery rate has little effect on the animal's willingness to use the system. Airflow through the GQS is an important factor, but as long as airflow is maintained above 26 L/s the breath-cloud capture seems nearly complete. There is great concern regarding circadian variation in the instantaneous production rates of CH_4 because the GQS normally only spot-samples 2 to 4 times/d. Preliminary analysis has shown that variation in the instantaneous production rates of CH_4 do not vary as greatly with grazing cattle compared with meal-fed cattle. It seems that increasing the visitation length decreases variation in estimated emissions, but there is a diminishing return to increasing visitation length. The GQS is a useful tool for researching the nutrition and emissions of grazing cattle, but great care must be taken to obtain the best quality data possible for use in this high-impact research.

Key words: carbon dioxide, greenhouse gases, methane, oxygen, ruminants

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INTRODUCTION

The concern over climate change and the accumulation of anthropogenic CO_2 and CH_4 in the atmosphere has sparked interest in mediating these gases' production, and the EPA states that among domestic animals, ruminants produce significant amounts of CH_4 through their digestive process (Shepherd, 2011). Globally, domestic ruminants are the largest emitters of CH_4, with approximately a third being produced in the United States (Shepherd, 2011; Herrero et al., 2013). Ruminants are significant sources of enteric CH_4 (Gill et al., 2009; Herrero et al., 2013) and contribute around 9% of the anthropomorphic greenhouse gas production. Since there are a myriad of factors that influence endemic CH_4 production, including diet digestibility, ruminal rate of passage, and DMI (responsible for approximately 70% of CH_4 variation) (Kennedy and Charmley, 2012; Charmley et al., 2016; Escobar-Bahamondes et al., 2016; Bond et al., 2017), emissions can represent energy losses varying from 2% to 12% of GE intake (Kujawa, 1994; Johnson and Johnson, 1995).

Since ruminant livestock are one of the few sources of CH_4 production we can manipulate, they represent the largest land-use system on Earth (Herrero et al., 2013), and they are a primary source of food security (Smith et al., 2012), this CH_4 source is an attractive target for reduction and its reduction is often associated with improved productivity (Leng, 1993). The collective environmental and economic benefits to the livestock industry and to society by decreasing livestock CH_4 emissions make research of factors impacting CH_4 production important (Johnson and Johnson, 1995). As a result of research efforts to decrease enteric CH_4 emission, systems are currently being developed to measure CH_4 emission by cattle in production environments. This research goal is often a challenge in open-air environments. A scientific instrument for this task is an automated, open-circuit gas quantification system (**GQS**) named GreenFeed and manufactured by C-Lock, Inc. (Rapid City, SD) that simultaneously measures CH_4 and CO_2 emitted and O_2 consumption by grazing ruminants. The GQS has similarities to open-circuit respiration chambers (McLean and Tobin, 1987), but it must sample the animal's breath cloud multiple times over several days to obtain a quality estimate of gas fluxes (Hegarty, 2013; Huhtanen et al., 2013; Arthur et al., 2017). The fact that the cattle select when and if they use the GQS, makes the management of these instruments challenging. In the discussion below, we outline some issues with management of grazing cattle, training, and programming the GQS.

Measuring Gas Flux Options for Grazing Cattle

An issue with grazing cattle research is the ability to measure gaseous emissions in open-air environments. This task was first addressed in the early 90s using an enteric CH_4 measuring system for grazing cattle that uses sulfur hexafluoride (SF_6) as a tracer gas (Zimmerman, 1993; Johnson et al., 1994). Zimmerman (1993) described the technique of using SF_6 as an inert tracer gas placed in the rumen, and it has been widely used (Johnson and Johnson, 1995). The release rate of the SF_6 gas through a permeation membrane is measured before the bolus containing the gas is inserted into the rumen. A halter fitted with a capillary tube is placed on the animal's head and connected to an evacuated sampling canister. As the vacuum in the sampling canister dissipates, a steady sample of the air near the mouth and nose of the animal is collected in the canister. After collection of a sample, the canister is pressurized with nitrogen, and then CH_4 and SF_6 concentrations are determined by gas chromatography or other available methods. Methane emission rate is then calculated as follows: $QCH_4 = QSF_6 \times [CH_4]/[SF_6]$, where QCH_4 is the emission rate of CH_4 in liters per hour, QSF_6 is the known release rate of SF_6 from the permeation membrane in the ruminal bolus, and $[CH_4]$ and $[SF_6]$ are the measured concentrations in the canister air. This technology was exciting to researchers because it eliminated the necessity to restrain or enclose the animal in a chamber; thus, allowing the animal to move freely and graze. Also, it was not necessary to sample directly from the animal's rumen or throat because the ability to use the tracer gas (SF_6) as an indirect marker. However, it is necessary to train the animal to wear the halter and collect the canisters, which is laborious. Also, this tracer technique does not measure all of the hindgut CH_4 production as would chamber systems. Any CH_4 from the hindgut that is absorbed into the blood stream will be expired and collected, but the CH_4 that escapes absorption and is released from the rectum is not collected and can account for 1% to 11% of the GE intake (McGinn et al., 2006; Murray et al., 2007). Additionally, the SF_6 system is integrative, so that diurnal variations of emissions are not measurable.

Due to the labor associated with the SF_6 system, the GQS (C-Lock, Inc.), as described by Hristov et al. (2015), has been developed to reduce labor requirements and speed analysis time. Also, if your research objectives require the measurement of CO_2, H_2, and H_2S emissions and O_2 consumption by grazing ruminants, the GQS is currently the only technology with the ability to measure these gases. Recent research has validated the GQS as accurately estimating CO_2 and CH_4 emission by cattle over extended periods of time (Huhtanen et al., 2013; Cottle et al., 2015; Dorich et al., 2015; Alemu et al., 2017).

Training Cattle to Use the GreenFeed Emission Monitoring System

The GQS is a head chamber that grazing cattle occasionally visit, and while the animal consumes a small portion of bait (6 to 8 allocations of 32 g each) over a 3- to 8-min period, the GQS captures the animal's breath cloud by exhausting air through the GQS. The breath cloud is immediately analyzed for CH_4, CO_2, and O_2 concentrations, and the data are stored onboard. Stored data are uploaded hourly to a server where it is processed using algorithms to determine total daily emissions and consumption of the gas of interest as described by Gunter et al. (2017) and Hristov et al. (2015) using ideal gas laws and mass airflow estimates. Several factors can influence the emission estimates generated by the GQS including the animal's visitation rate, length of sampling period, and airflow through the GQS.

The location of the GQS is an important factor in determining the cattle's willingness to visit the GQS. The GQS must be in areas where the cattle are willing to visit frequently. The GQS itself, even though it uses feed as bait to entice the animal to use it, is not a strong enough enticement to attract cattle to use it at distant locations in a large pasture, like research has demonstrated with salt and supplements (Bailey and Welling, 1999; Ganskopp, 2001). In our research, experimental pastures range from 20 to 40 ha and cattle graze as far as a kilometer from the GQS. At the Southern Plains Experimental Range, we have our GQS positioned near the only water source for the 40-ha pastures (Figure 1). Because it has been demonstrated that cattle remain near water sources (Valentine, 1947; Owens et al., 1991), the cattle will walk by the GQS 2 to 3 times a day as they travel to and from the water supply.

Figure 1. An automated, open-circuit gas quantification system (GreenFeed; C-Lock, Inc.) located at the Southern Plains Experimental Range near Fort Supply, OK.

Training cattle to use the GQS long before beginning the experiment is the most important step to a successful outcome. Some cattle will never use the GQS, so a researcher needs to start with at least 20% to 30% more subjects at the beginning of the training period so animals that never use the GQS can be removed. Cattle training needs to start more than 4 wk before the start of an experiment (up to 8 wk of training may be necessary), especially when cattle are in a grazing environment. During the first week of training, the cattle should be penned with the GQS located adjacent to the water tank, with a supply of grass hay. Then, as the cattle use the GQS more frequently during week 2 through 4, the cattle can be allowed out to pasture during the day for increasing durations, and by the end of the fourth week, they may have continuous access to the pasture and hay can be withdrawn. Even in a semi-arid environment like northwest Oklahoma, cattle will not water every day during the spring when new forage emerges that is high in moisture. Hence, cowboys may need to drive the cattle back to the GQS and water source on occasion to maintain an acceptable frequency of emissions sampling. This long training period is time consuming, but it is necessary for the cattle to use it regularly for the attainment of quality gas consumption and emission estimates (Arthur et al., 2017; Gunter and Bradford, 2017).

Bait Delivery and Cattle Use

Once cattle are trained to use the GQS, the bait delivery rate has little effect on willingness to use the GQS and gas flux estimates, as long as visitation length is greater than 3 min (Arthur et al., 2017). Two experiments by Gunter and Bradford (2017) demonstrated that when the GQS is programmed to sample cattle every 4.5 h and to allow 8 aliquots of bait per visit with bait delivery rates intervals between 18 and 43 s (sampling period of 43 to 73 d), CO_2 emission estimates did not differ among bait delivery intervals (Table 1). But, the CH_4 emission estimates and the ratio of CH_4:CO_2 linearly decreased ($P < 0.01$) with increasing time increment in the first experiment, but not the second. In the second experiment, time increment did not affect the O_2 consumption estimate. Hence, cattle did not respond consistently to increasing time increment among bait deliveries, but bait delivery interval had minimal effect on overall gas emission and consumption estimates.

Airflow Rate in the GreenFeed Emission Monitoring System

Airflow through the GQS is an important factor for the GQS to capture the breath cloud completely for an accurate estimate and is managed by keeping a clean air filter in the GQS. This goal is achieved when the airflow is at or above the manufactures recommended rate of 26 L/s. A retrospective analysis was conducted by Gunter et al. (2017) with a dataset where breath samples were collected and analyzed for CO_2 and CH_4 with airflow rates that ranged from 10.7 to 36.6 L/s with an average airflow rate of 24.3 (Table 2). Carbon dioxide and CH_4 emission rates measured during these sampling events were fitted to a linear mixed-effects model. The full model considered in the analysis included fixed effects for airflow, airflow squared, and day of study. Day of study was included because gas emission rates may not have been constant during the entire sampling period because of changes in animal BW and herbage

Table 1. Effect of bait delivery interval on gas flux estimates by grazing beef heifers (BW = 364 ± 2.4 kg) in two experiments using an automated, open-circuit gas quantification system (GreenFeed; C-Lock, Inc.; Gunter and Bradford, 2017)

	Bait delivery interval in Exp. 1 [a], s					Contrast	
Item	18	21	24	27	SE	L	Q
Daily Mass, g							
CO_2 emitted	5,675	5,882	5,515	5,771	66.5	0.77	0.63
CH_4 emitted	158	169	138	151	4.1	<0.01	0.68
CH_4/CO_2, M/M	0.076	0.076	0.068	0.071	0.002	<0.01	0.35
	Bait delivery interval in Exp. 2 [a], s						
	19	27	35	43	SE		
Daily mass, g							
CO_2 emitted	4,983	5,325	4,921	5,029	120.4	0.44	0.11
CH_4 emitted	159	166	157	160	6.8	0.79	0.64
O_2 consumed	4,131	4,496	4,146	4,119	121.4	0.27	<0.01
CH_4/CO_2, M/M	0.089	0.089	0.088	0.090	0.0033	0.77	0.79

[a] Seconds between 32-g increments of bait (alfalfa pellets, 6.25-mm diameter) delivered to heifers.

Table 2. Fixed and random effects coefficients from mixed models fit to two different ranges in airflow rates through an automated, open-circuit gas quantification system (GreenFeed; C-Lock, Inc.; Gunter et al., 2017)

		Fixed effect[a]			Random effect[b]		
Gas, g/d	Transformation	Intercept	cFlow	cDOS	Intercept	cDOS	Residual
26.0 L/s ≤ airflow ≤ 36.6 L/s[c]							
CO_2	None	4,316.8	-	31.08	284.0	-	569.9
CH_4	Box-Cox[d]	22.429	-	0.0455	1.024	0.055	2.938
10.7 L/s ≤ airflow < 26.0 L/s[e]							
CO_2	None	4,006.1	53.77	23.27	316.2	-	656.9
CH_4	Box-Cox	21.205	0.2493	-	1.177	-	2.732

[a] Fixed-effect terms center airflow rate (cFlow) and center day of the study (cDOS) are centered on airflow (L/s) and day of study (d), respectively, by centering their mean value across the entire data set (cFlow = Flow − 24.26, cDOS = DOS – 25.90), so the intercept can be interpreted as the response when airflow and day of study are at their mean values and DOS is day of study.

[b] Random effect terms are SD of Intercept and cDOS, grouped by heifer (n = 13), and residual SD of within-heifer error.

[c] The manufacturer's recommended airflow rate range (S. Zimmerman, C-Lock, Inc., personal communication).

[d] Box-Cox transformed data, airflow rate = $g/d^{0.5-1}$.

[e] Less than the manufacturer's recommended airflow rates (S. Zimmerman, C-Lock, Inc., personal communication).

maturities. Further, the data were divided into two datasets: the first had airflows ≥26.0 L/s and the second had airflows <26.0 L/s. When using records in the analysis where airflow was ≥26 L/s, there was a significant day-of-study effect on both CO_2 and CH_4 emissions (Table 2), but no significant airflow rate effect. When limiting the dataset to only those records where airflow rate was <26 L/s, there was a significant effect of airflow rate on estimated CO_2 and CH_4 estimates (Table 2). Also, day of study had a significant effect on CO_2, and the CO_2 emission equation describing this lower range of data showed that as airflow rates decreased, CO_2 emission estimates decreased (Table 2). Last, the CH_4 emission estimates developed with airflow data ranging from 10.7 to 26.0 L/s did not vary with day of study, but did decrease as airflow rate decreased. The Box-Cox transformed CH_4 emissions decreased 0.249 units for each L/s decrease in airflow rate (unit = $g/d^{0.5-1}$). Back-transforming to the population level results at 25 L/s, the calculated CH_4 emission is 136.8 g/d, and at 15 L/s, the calculated CH_4 emission is 109.1 g/d, a decrease of 27.7 g/d.

The GQS uses the same principles of gas flux for measuring gas emissions as respiration chambers where an active airflow is induced to capture the emitted breath (Huhtanen et al., 2015), and the GQS integrates measurements of airflow, gas concentrations, and the detection of muzzle position to allow for the direct measurement of gas fluxes during the animal's visit. This interaction among multiple measurements and results of Gunter et al. (2017) shows the importance of capturing as much of the emitted breath as possible. The reason for this decrease in CO_2 and CH_4 emissions when airflow is less than 26 L/s is likely the result of leakage of the animal's emitted breath cloud from the GQS. Eructation of ruminal gas is injected into the tidal-breath cloud at intervals of 40 to 60 s (Van Soest, 1994; Caetano et al., 2017) depending on environmental (Webster et al., 1970; McArthur, 1987) and physiological (Young, 1966; Osuji, 1974) factors. Given the differences in release timing and point, and differences in gas densities, it is reasonable to believe that the eructation cloud and tidal-breath cloud would not be uniformly mixed (Judd et al., 1999). Many researchers recommend using only spot-samples with duration longer than 3 min (Velazco et al., 2014; Huhtanen et al., 2015; Arthur et al., 2017) to capture an adequate number of eructation events within the tidal-breath cloud and reduce the variation among CH_4 emission estimates. Unlike CO_2 emissions, which are emitted in a more constant manner, CH_4 is emitted as pulses into the emission cloud that vary in number, volume, and concentration. This irregular pattern of CH_4 emissions is mostly responsible for CH_4 emission estimate data that are not normally distributed. With the GQS, a large airflow rate induced into the feed pan creates an airflow field that more completely captures the emitted tidal breath. Hence, maintaining sufficient airflow rate through GQS is imperative to capture the complete tidal breath of the animal.

Circadian Variation in Instantaneous Gas Emissions

There is great concern regarding circadian variation in the instantaneous production rates of CH_4

because the GQS usually only spot-samples 3 to 4 times/d depending on the researcher's programming (McCrabb and Hunter, 1999; Hegarty, 2013; Alemu et al., 2017). With meal-fed cattle (i.e., feedlot systems), the instantaneous production rates of CH_4 differ greatly across the day. For example, research by Hales and Cole (2017) showed that when cattle were fed a steam-flaked, corn-based diet once daily, at 2 times maintenance, the instantaneous production of CH_4 ranged from a low of 1.4 L/h an hour before feeding to a high of 4 L/h occurring 5 h after feeding, a 160% increase. However, an analysis has shown that the instantaneous production rates of CH_4 do not vary as greatly with grazing cattle. Gunter and Bradford (2015) analyzed 2,377 records harvested with a GQS with stocker cattle grazing mixed-grass prairie in northwest Oklahoma from November through January. Analysis for a circadian variation in the instantaneous production rates of CO_2 and CH_4 within these data showed that the instantaneous production of CO_2 and CH_4 ranged from a low of 117 and 12 L/h, respectively, during the late morning (0600 to 1159 h), to a high of 129 and 14 L/h, respectively, in the evening (1800 to 2359 h), only a 16% increase. Hence, CO_2 and CH_4 emissions from grazing cattle were affected by a circadian emission pattern, but the variation was much less pronounced than with meal-fed cattle (Hammond et al., 2013; Gunter and Bradford, 2015). Hence, with grazing cattle, the range in hourly CO_2 and CH_4 emission rates during the day is greatly reduced compared with meal-fed cattle, so the use of "snapshot" sampling to estimate daily CO_2 and CH_4 emissions probably gives an accurate estimate when animals have *ad libitum* feed available and sampling is distributed throughout the day. However, this issue of circadian variation needs to be fully explored with more robust datasets from animals grazing different forage types (species and qualities) and allowances.

Record Number to Achieve a Quality Estimate

The last issue to be discussed is the number of records required to achieve a high nominal power for the estimate. Gunter and Bradford (2017) used a power analysis with cattle visiting the GQS approximately 2.4 times a day; they reported that between 3.4 and 3.8 d of measurements was required to quantify CO_2 emissions with an α value of 0.01 and a nominal power of 90%, required between 4.8 and 6.3 d to quantify CH_4 emissions accurately, and required between 3.7 and 4.1 d to quantify O_2 consumption accurately. These estimates of required record numbers by Gunter and Bradford (2017) are less than reported by Renand and Maupetit (2016) where the time required to achieve a quality estimate of CH_4 emission was a period of about 2 wk (containing approximately 50 spot measurements) per animal. Arthur et al. (2017) reported a steep drop in variance at approximately 30 records, beyond which there were no substantial reduction in variance. Last, researchers from France (Arbre et al., 2016) showed that in order to achieve a repeatability of 0.70 for the CH_4 estimate a 17-d period was required. Hence, based on the considerable research conducted regarding the number of records required for a high level of repeatability, it seems that most research suggests 30 to 50 records.

The GQS is a useful tool for researching the nutrition and emissions of grazing cattle, but this system necessitates certain considerations to produce and obtain the best quality data possible for this high-impact research. For cattle in open-air environments like when grazing, the SF_6 and GQS are the only two systems available. The SF_6 procedure (Zimmerman, 1993) produces quality estimates of CH_4 production, but the GQS (Hristov et al., 2015) also allows a scientist to measure CO_2 and other minor gas emissions, and O_2 consumption. Great care must be taken to monitor the animals' use of the GQS and that the system is operating with in manufacture's recommendation, like airflow and calibrations (Gerrits et al., 2017), so the emission and consumption estimates have a high nominal value. Circadian variation of instantaneous gas emission and consumption rates is of concern; however, because cattle are eating intermittently throughout the day (grazing), the magnitude of changes in instantaneous gas emission and consumption rates is smaller than with meal-fed cattle and sampling with the GQS occurs throughout the day when cattle opt their times of visitation to the GQS. If the GQS is operating correctly, quality estimates of CO_2 and CH_4 emission and O_2 consumption are obtainable when sampling for approximately a 14-d period when animals are visiting the GQS more than 2.5 times per day.

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