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Technical note: using an automated head chamber system to administer an external marker to estimate fecal output by grazing beef cattle

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Abstract

The objective of this experiment was to determine if titanium dioxide (TiO_2) dosed through an automated head chamber system (GreenFeed; C-Lock Inc., Rapid City, SD, USA) is an acceptable method to measure fecal output. The GreenFeed used on this experiment had a 2-hopper bait dispensing system, where hopper 1 contained alfalfa pellets marked with 1% titanium dioxide (TiO_2) and hopper 2 contained unmarked alfalfa pellets. Eleven heifers (BW = 394 ± 18.7 kg) grazing a common pasture were stratified by BW and then randomized to either 1) dosed with TiO_2-marked pellets by hand feeding (HFD; *n* = 6) or 2) dosed with TiO_2-marked pellets by the GreenFeed (GFFD; *n* = 5) for 19 d. During the morning (0800), all heifers were offered a pelleted, high-CP supplement at 0.25% of BW in individual feeding stanchions. The HFD heifers also received 32 g of TiO_2-marked pellets at morning feeding, whereas the GFFD heifers received 32 g of unmarked pellets. The GFFD heifers received a single aliquot (32 ± 1.6 g; mean ± SD) of marked pellets at their first visit to the GreenFeed each day with all subsequent 32-g aliquots providing unmarked pellets; HFD heifers received only unmarked pellets. Starting on d 15, fecal samples were collected via rectal grab at feeding and every 12 h for 5 d. A two-one sided t-test method was used to determine agreement and it was determined that the fecal output estimates by HFD and GFFD methods were similar ($P = 0.04$). There was a difference ($P < 0.01$; Bartlett's test for homogenous variances) in variability between the dosing methods for HFD and GFFD (SD = 0.1 and 0.7, respectively). This difference in fecal output variability may have been due to variability of dosing times-of-day for the GFFD heifers (0615 ± 6.2 h) relative to the constant dosing time-of-day for HFD and constant 0800 and 2000 sampling times-of-day for all animals. This research has highlighted the potential for dosing cattle with an external marker through a GreenFeed configured with two (or more) feed hoppers because estimated fecal output means were similar; however, consideration of the increased variability of the fecal output estimates is needed for future experimental designs.

Key words: beef heifers, fecal output, GreenFeed, titanium dioxide

Abbreviations:

CH_4	carbon dioxide
GreenFeed	automated head chamber system
TiO_2	titanium dioxide

Introduction

Concern for the environment and the impact of livestock production on climate change is increasing. Scientists have estimated that 14.5% of all anthropogenic greenhouse gas emissions come from global livestock production (animals, manure, feed production, and expansion of lands into forested areas) and, of that, 39% results from enteric methane (CH_4) emissions (Gerber et al., 2013). Further, cattle grazing pastures in North American emitted in excess of 85% of all enteric CH_4 (Beauchemin et al., 2010). As total enteric CH_4 emissions are highly correlated with DM intake (Robinson et al., 2010; Charmley et al., 2016), there is a renewed interest in accurately estimating DM intake of grazing cattle when assessing their CH_4 yield (g of CH_4/kg of DMI; Beauchemin et al., 2010; Alemu et al., 2017; Shreck et al., 2021).

The sulfur hexafluoride method (Muñoz et al., 2012) has been successfully used to measure CH_4 emissions from grazing ruminants, but more recently, the spot gas sampling technique (GreenFeed) has been adopted by many researchers (Hristov et al., 2015; Gunter and Beck, 2018; McGinn et al., 2021). The GreenFeed provides a pelleted supplement as bait to entice animals to visit the unit, which captures and analyzes the composition of their breath cloud. Further, the predominate method used to measure DM intake by grazing cattle is the dual marker method described by Kartchner (1980), which involves dosing cattle with an external marker, such as TiO_2 (Myers et al., 2006), to estimate fecal output and then using internal markers to estimate herbage digestibility (Judkins et al., 1990).

If TiO_2 can be successfully dosed by the GreenFeed baiting system to create a steady state of marker excretion, several benefits could be realized—that is, reduced labor, increased experimental units, and reduced grazing behavior disruption. We hypothesized that there would be adequate agreement in fecal output estimated using TiO_2 as an external marker dosed between the GreenFeed and handfeeding in stanchions.

Materials and Methods

All animal procedures were conducted in accordance with the recommendations of the Consortium (FASS, 2010) and were approved by the Southern Plains Range Research Station Institutional Animal Care and Use Committee (Protocol Number AUP-008).

This study was conducted from 4 February to 23 February 2017 (19 d) at the Southern Plains Experimental Range (36°35′ N, 99°35′ W; elevation 630 m) of the USDA, Agricultural Research Service near Fort Supply, OK, USA. The regional climate is continental, an average annual precipitation of 627 mm, with 72% of the precipitation falling during the April to September growing season (Gunter et al., 2012). Average monthly mean temperatures are 2.3°C in January and 28°C in July. This region consists of gently rolling and stabilized sand dunes and the vegetation is dominated by a mixture of tall, mid, and short native warm-season grasses and forbs interspersed among sand sagebrush (*Artemisia filifolia*) plants. The CP, NDF, ADF, and the in vitro organic matter digestibility concentrations in herbage samples collected from this site in mid-February averaged over multiple years are 5.0% ± 0.36, 73.0% ± 0.20, 49.0% ± 0.33, and 36.7% ± 0.34 (mean ± standard error) of DM, respectively (Savage and Heller, 1947; Gadberry et al., 2012; Gunter, 2019).

Red Angus heifers (BW = 394 ± 18.7 kg; mean ± SD; n = 12) were stratified by BW and allotted to treatments of either 1) dosed with TiO_2 through the GreenFeed (GFFD) or 2) dosed with TiO_2 when hand-fed (HFD). All heifers were supplemented with a high-protein, oil-seed meal-based supplement (Table 1) during the morning (0800), in individual feeding stanchions, at 0.25% of BW. The HFD heifers received an additional 32 g of TiO_2-marked pellets (1% TiO_2; Table 1) and the GFFD heifers received an additional 32 g of unmarked alfalfa pellets (Table 1) at each feeding during the 19-d experiment. The GreenFeed used during this experiment is fitted with the 2-hopper feeding system (www.c-lockinc.com). Hopper 1 contained the unmarked pellets (30.08 ± 3.0 g/aliquot; mean ± SD) and hopper 2 contained the TiO_2-marked pellets (32.48 ± 1.6 g/aliquot; mean ± SD). The GreenFeed was set to allow 4 visits per day with 4.5 h between visits and to drop aliquots of feed 8 times/visit with 30-s intervals between aliquots to achieve at least 3-min sampling periods (Gunter and Bradford, 2017) and at various periods of the day (Robinson et al., 2010; Gunter and Bradford, 2015). Hence, the GFFD treatment received their first bait aliquot from hopper 2 containing TiO_2-marked pellets during their first daily visit and then received seven aliquots of unmarked pellets from hopper 1. The HFD heifers only received unmarked pellets (hopper 1), while visiting the GreenFeed.

The GreenFeed used was constructed to measure CO_2 and CH_4 emissions and O_2 consumption. The GreenFeed uses the average of intermittent measurements to determine average daily CO_2 and CH_4 production (g/d) and O_2 consumption (g/d) using pelleted feed as bait as described in detail by Hristov et al. (2015). In short, when an animal places its head into the GreenFeed hood, its radio frequency identification tag is read and stimulates the bait to be dispensed. While the animal is eating, a fan draws air around the animal's muzzle to capture the respiratory gas cloud emitted. A subsample of the captured breath cloud is harvested and analyzed by sensors for CO_2, CH_4, and O_2 concentrations. The respiratory gas concentrations in the captured gas are related to the respective gas concentrations in the ambient air drawn in before and after the animal visited and the emissions and consumption by the animal can be determined by difference as described by Gunter et al. (2017).

Table 1. Chemical characteristics of feeds and supplements used in the experiment near Ft. Supply, Oklahoma

	Diet and supplements		
Item, % DM	Unmarked pellets[1]	Marked pellets[2]	Supplement[3]
CP	14.8	17.9	42.8
GE, Mcal/kg	4.1	4.1	4.3
NDF	23.2	48.2	19.0
ADF	12.5	33.4	12.2
IVOMD	87.5	73.1	89.2

[1]Unmarked Pellets, GreenFeed bait that did not contain titanium dioxide.
[2]Marked Pellets, GreenFeed bait that contained 1% titanium dioxide.
[3]Supplement, pelleted supplement provided to all animals at 0.25% of BW/d in individual supplementation stanchions.

Starting the morning of d 15 (0 h), fecal samples were taken from each heifer, via rectal grab, and every 12 h for 5 d (12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 h). Fecal samples were then dried in a 60°C forced-air oven and ground to pass through a 2-mm screen (Model 4 Thomas A. Wiley Laboratory Mill; Thomas Scientific, Swedesboro, NJ, USA). Fecal samples were composited, by equivalent weight, for each animal for the 120-h period. These composited samples were then thoroughly mixed and analyzed for Ti by X-ray fluorescence spectrometry (Barnett et al., 2016; Hoffmann et al., 2020). Fecal DM output (FO; kg/d) from Ti was calculated as the ratio of the amount of Ti dosed divided by the concentration (g Ti/kg DM) in the feces (Kartchner, 1980), using the Ti concentration values from the composited fecal samples:

$$\text{Fecal output}\left(\frac{\text{kg DM}}{\text{day}}\right)=\frac{\text{Ti dosed}\left(\frac{\text{g Ti}}{\text{day}}\right)}{\text{Ti in feces}\left(\frac{\text{g Ti}}{\text{kg feces}}\right)}.$$

Statistical analysis of results

Method comparison of the TiO_2 dosing techniques (GFFD vs. HFD) were conducted using the two-one sided t-test (TOST) method to determine agreement and a t-test to compare statistical differences (null hypothesis statistical test; NHST). These tests were conducted using the "TOSTER" package of R (Lakens, 2017). Finally, an a posteriori power analysis was conducted based on the observed variabilities determined for the GFFD and HFD treatment groups using the "power.anova.test" function assuming a power of 0.80, a significance level of $P = 0.05$, and a 10% difference between treatments. All statistical analysis was conducted using R (R Core Team, 2017).

Results

One heifer from the GFFD treatment was removed from the Ti marker analysis due to unrealistic estimates. The heifer had an estimated 1.1 kg/d fecal output. This was due to an abnormally high concentration of Ti in the feces (0.14%), which was seven times greater than her contemporaries. The reason for this is unclear, but is obviously unrealistic, and must be due to an extraneous error. Hence, the estimate for this animal was removed from consideration.

The CH_4 and CO_2 emissions and O_2 consumption by the cattle did not differ between treatments ($P \geq 0.17$), but the TOST analysis showed the three gas fluxes were not significantly similar ($P \geq 0.11$). Initial BW were not different between treatments ($P = 0.85$) and were similar between treatments ($P < 0.01$; Table 2) per design. During the 19-d experimental period, average daily gain did not significantly differ ($P = 0.24$) between HFD and GFFD treatments and were not significantly similar ($P = 0.84$). After the experimental period, final BW did not differ ($P = 0.85$) between HFD and GFFD treatments and were similar ($P = 0.01$; Table 2). The marker dosing methods (GFFD vs. HFD) used did not affect ($P = 0.43$) the fecal output estimates and the methods were statistically similar ($P = 0.04$; Table 2). There was a difference ($P < 0.01$; Bartlett's test for homogenous variances) between HFD and GFFD in the variation noted in fecal output (SD = 0.1 and 0.7, respectively). It was determined according to an a posteriori power analysis that three and seven replicates would be needed using the variability of the HFD and GFFD heifers, respectively.

Discussion

The fecal output (DM basis) by the heifers in this experiment was 2.0% and 1.9% of BW for the HDF and GFFD heifers, respectively. As a percentage of BW, these fecal DM output estimates are like other reports (Gunter et al., 1997; Johnson et al., 1998). We did identify a difference in fecal output variation between HFD and GFFD dosing methods. We speculate that this increased variation associated with the GFFD method is related to the fact that the first visit time to the GreenFeed each morning was ad libitum. The intake of the titanium dose from the GreenFeed by the GFFD heifers occurred over an extended period each morning, whereas the HFD method was dosed at a relatively consistent time each morning. On average the GFFD treatment had their first visit at 0615 ± 6.2 h (mean ± SD). An increased variability may necessitate a greater sample size, increased sampling events, or both, when dosing animals with an external marker through the GreenFeed. Diel variation in fecal marker concentration has long been known to be present when dosing ruminants once or even multiple times per day (Kiesling et al., 1969; Nelson and Green, 1969; Sampaio et al., 2011), and several methods of dosing and sampling schemes have been employed to minimize the occurrence and effect of variation of marker concentrations in the feces on fecal output estimates with limited success (Pigden and Brisson, 1956; Brisson et al., 1957; Abdouli et al., 1992). Despite the GFFD heifers having a seven times greater fecal output SD than the HFD heifers, the GFFD

Table 2. Animal responses, fecal output, and gas flux as effected by indigestible marker dose method in the experiment near Ft. Supply, Oklahoma

	Treatment[1]		P-value[2]	
Item (± equivalence bound)	GFFD (SD)	HFD (SD)	TOST	NHST
n	5	6	–	–
CO_2, g/d (± 479.4)	4,937.9 (325.2)	4,649.1 (309.8)	0.18	0.17
CH_4, g/d (± 13.0)	136.2 (13.9)	124.6 (33.1)	0.47	0.46
O_2, g/d (± 415.0)	4,235.2 (314.5)	4,065.6 (359.7)	0.11	0.39
Initial BW, kg (± 39.5)	393.5 (21.0)	395.8 (17.8)	<0.01	0.85
Average daily gain, kg/d (± –0.06)	–0.5 (0.3)	–0.7 (0.2)	0.81	0.24
Final BW, kg (± 38.2)	383.2 (25.5)	380.5 (19.6)	0.01	0.85
Fecal DM output, kg/d (± 1)[3]	7.4 (0.7)	7.1 (0.1)	0.04	0.43

[1]GFFD, GreenFeed fed; HFD, hand fed.
[2]TOST, two-one sided t-test; NHST, null hypothesis statistical test (t-test).
[3]Fecal DM output determined by titanium dioxide as external marker. The a priori equivalence bound was set at 1 kg/d for fecal output and at 10% of the treatment mean for all other variables.

heifers still had only a 9.5% CV. Although this variation displayed by GFFD heifers is not excessive, the HFD heifers had a 1.4% CV. Hence, more research is required to determine the effect of marker dosing-time variability on fecal output determination and this fact must be considered when designing an experiment.

The estimate of respiratory CO_2 emission in this experiment (4,794 g/d) are similar to estimates by Chaves et al. (2006) with yearling heifers (BW = 380 ± 9.8 kg, ± SD; 6,633 g/d) grazing meadow brome (*Bromus biebersteinii*) pastures and Gunter and Bradford (2017) with heifers grazing dormant mixed-grass prairie (BW = 364 ± 2.4 kg, ± SD; 5,711 g/d). The similarity among the estimates of Chaves et al. (2006), Gunter and Bradford (2017), and Shreck et al. (2021) and our experiment is likely because CO_2 emission is related to DM intake and intake would be similar on these sites and classes of cattle (Robinson et al., 2010; Charmley et al., 2016). The mean enteric CH_4 emissions (130 g/d) in our experiment are similar to estimates reported by Chaves et al. (2006) with yearling heifers grazing meadow brome pastures (144 g/d), Gunter and Bradford (2017) with heifers grazing dormant mixed-grass prairie (161 g/d), and Shreck et al. (2021) with steers fed big bluestem (*Andropogon gerardii* Vitman) hay. Further, the estimates of O_2 consumption averaged 11.1 g of O_2/kg of BW per day for the non-fasting heifers is greater than the 6.9 g of O_2/kg of BW per day for fasting dairy cows (Blaxter and Wainman, 1966), but is quite similar to the O_2 consumption rate (11.9 g/kg of BW per day) for steers consuming orchardgrass (*Dactylis glomerata* L.) silage (Huntington et al., 1988) and for heifers grazing dormant mixed-grass prairie (10.9 g of O_2/kg of BW per day; Gunter and Bradford, 2017). Expressing the average CO_2 and CH_4 emissions (g) and O_2 consumption in this experiment on a kilogram of BW per day basis (12.5 g, 1.34 g, and 10.9 g, respectively) compared closely to values reported by Gunter and Bradford (2017) for CO_2 (13.9 g/kg of BW) and CH_4 (0.44 g/kg of BW) emission, and O_2 (11.6 g/kg of BW) consumption. The respiratory quotient (RQ) for the heifers in this experiment averaged 0.84 (mol CO_2/mol CH_4) and is within the suggested normal range of 0.70–1.0 (Kleiber, 1961) and is similar to the RQ values for growing cattle grazing similar swards (Aubry and Yan, 2015; Gunter and Bradford, 2017).

The cattle in the experiment lost BW at a rate of 0.7 kg/d during this experimental period (19 d), and Aiken and Tabler (2004) have demonstrated that BW gain and retained energy estimates by cattle are difficult to assess during short observation periods (<60 d) so the actual rate of energy retention or loss is unknown. Additionally, because molecules that are more oxidized (e.g., glucose) require less oxygen to be fully metabolized, they have a higher RQ. Molecules that are less oxidized, such as VFA, require more oxygen for their complete metabolism and have a lower RQ (Kleiber, 1961). The RQ in fasting sheep for acetic, propionic, and butyric acids is 1.00, 0.86, and 0.80, respectively (Armstrong and Blaxter, 1957). As the normal VFA composition in cattle grazing dormant prairie is approximately 77, 13, 8, and 2 mol/100 mol for acetic, propionic, butyric, and branch-chain fatty acids, respectively (McCollum et al., 1987), cattle grazing these dormant rangelands have a RQ less than one. Hence, herbage intake by the heifers in our experiment seems to be within normal ranges based on the respiration parameters and should result in normal gastrointestinal function. The gas emission and body weight results lend support for the similar fecal outputs determined between GFFD and HFD heifers because respiratory gas emission and consumption is highly related to dry matter intake (Robinson et al., 2010; Charmley et al., 2016).

Conclusions

Based on these results, researchers can dose indigestible external markers, such as TiO_2, to grazing cattle that are being monitored for respiratory gas emissions by the GreenFeed. With the fecal output estimates being nearly identical between delivery methods, we conclude that delivering an external marker through the GreenFeed provides an unbiased estimate. However, there was a larger SD for the fecal output estimates when ruminants were dosed by the GreenFeed compared with HFD. According to an a posteriori power analysis, this larger variability can be compensated for with increased replication per treatment, but it also may be overcome by increasing sampling events to account for diel variation.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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