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Short Communication

# Comparative methane estimation from cattle based on total CO₂ production using different techniques

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## ABSTRACT

The objective of this study was to compare the precision of $CH_4$ estimates using calculated $CO_{2\,(HP)}$ by the $CO_2$ method ($CO_2T$) and measured $CO_2$ in the respiration chamber ($CO_2R$). The $CO_2R$ and $CO_2T$ study was conducted as a $3 \times 3$ Latin square design where 3 Dexter heifers were allocated to metabolic cages for 3 periods. Each period consisted of 2 weeks of adaptation followed by 1 week of measurement with the $CO_2R$ and $CO_2T$. The average body weight of the heifer was $226 \pm 11$ kg (means $\pm$ SD). They were fed a total mixed ration, twice daily, with 1 of 3 supplements: wheat (W), molasses (M), or molasses mixed with sodium bicarbonate (Mbic). The dry mater intake (DMI; kg/day) was significantly greater ($P < 0.001$) in the metabolic cage compared with that in the respiration chamber. The daily $CH_4$ (L/day) emission was strongly correlated ($r = 0.78$) between $CO_2T$ and $CO_2R$. The daily $CH_4$ (L/kg DMI) emission by the $CO_2T$ was in the same magnitude as by the $CO_2R$. The measured $CO_2$ (L/day) production in the respiration chamber was not different ($P = 0.39$) from the calculated $CO_2$ production using the $CO_2T$. This result concludes a reasonable accuracy and precision of $CH_4$ estimation by the $CO_2T$ compared with the $CO_2R$.

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## 1. Introduction

Methane ($CH_4$) is a byproduct of rumen fermentation produced by methanogenic archaea. Methanogens use hydrogen ($H_2$) in the rumen to produce $CH_4$. Thus, they keep $H_2$ pressure low which favors anaerobic fermentation of ingested materials. Cattle are some of the main contributors of anthropogenic $CH_4$ gas emissions to the atmosphere (Gerber et al., 2013). This particular greenhouse gas has received a great deal of attention in the recent years not only because of its involvement in global warming processes leading to climate change, but also because it represents a loss of energy from the animals. Typically, methane emissions are about 2% to 12% of the gross energy intake depending on e.g., roughage-to-concentrate ratio in the feed, carbohydrate composition and use of supplements and additives (Johnson and Johnson, 1995). Enteric $CH_4$ production is a process very closely related to the composition of the volatile fatty acids produced in the rumen (Johnson and Johnson, 1995). The primary substrate for methanogenesis is $H_2$ that is generated during fermentation of plant cell wall carbohydrates. The products of this fermentation are primarily acetate and butyrate (Moss et al., 2000). Fermentation of starch and other non-structural carbohydrates favor propionate production. Propionate production is a competitive pathway for $H_2$ use in the rumen (Benchaar and Greathead, 2011). Unlike starch, fermentation of sugar by rumen microbes has been reported to increase methane production (Hindrichsen et al., 2004). Rumen microbial fermentation of sugar leads to a preferential production of butyrate at the expense of propionate (Friggens et al., 1998), hence, results higher methane production.

The respiration chamber was the only method for methane estimation from cattle for hundreds of years. Currently, several methods have been developed to estimate the actual emissions from livestock. They are based on different principles and have a wide range of optimal applicability (Storm et al., 2012). One of the methods with a wide applicability, the $CO_2$-method ($CO_2T$) is described by Madsen et al. (2010). The $CO_2T$ uses the total $CO_2$ production from the animal as a marker for $CH_4$ estimation. The

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hypothesis of this study was that the precision of the $CH_4$ estimates by the $CO_2T$ (using calculated total $CO_2$) would be comparable with a reference method ($CO_2R$; using measured total $CO_2$). Therefore, the present study was designed to compare the precision of $CH_4$ estimates between the $CO_2T$ and $CO_2R$ technique.

## 2. Materials and methods

### 2.1. Experimental design, animals and feeding

This present study was conducted with a 3 × 3 Latin square design where 3 Dexter heifers were allocated to balance cages for 3 periods consisting of 2 weeks of adaptation followed by 1 week of measurement. The animals were weighed at the start and end of the experiment. The average body weight (BW) of the heifers was 226 ± 11 kg (means ± SD) and the average dry matter intake (DMI) was 5.1 ± 0.3 kg/day (means ± SD) throughout the entire experiment. The animals were fed twice daily with a total mixed ration (TMR) made up (on DM basis) of 49% grass-clover silage, 14% soybean meal along with 35% of 1 of 3 supplements: wheat (W), sugar beet molasses (M), or sugar beet molasses mixed with sodium bicarbonate (Mbic) as a buffer to prevent low rumen pH. All feed for the entire experiment was prepared once from the same batches of ingredients. After preparation, daily portions of the TMR were immediately vacuum-packed and frozen. Each portion was thawed at room temperature overnight before being fed *ad libitum* twice daily. The chemical composition of the diets is shown in Table 1. The daily feed intake was measured by the difference between the amount of supply and orts.

### 2.2. Measurement techniques

#### 2.2.1. $CO_2$-technique

Breath samples from the heifers were continuously measured every 20 s for 3 days (1 day at a time for each diet) in the metabolic cage to analyze the concentrations (parts per million) of $CH_4$ and carbon dioxide ($CO_2$). A portable continuous gas analyzer GASMET DX-4030 (Gasmet Technologies Oy, Helsinki, Finland) was used to analyze the breath concentrations based on Fourier Transformed Infrared (FTIR) detection. The metabolic cages were placed in a restricted ventilated barn which was kept open during the day time. The gas sampling inlet was attached to the metabolic cage, at the nose level of the heifers. The recorded concentrations of breath samples were stored in a data logger on a computer. Baseline barn air concentration was measured for 10 min during each experimental

**Table 1**
Dietary and chemical composition of 3 diets.

| Item | W | M | Mbic |
|---|---|---|---|
| Composition of the ration, g/kg DM | | | |
| Grass-clover silage | 494 | 494 | 490 |
| Wheat | 353 | | |
| Sugar beet molasses | | 353 | 350 |
| NaHCO$_3$ | | | 9.3 |
| Soybean meal | 141 | 141 | 140 |
| Mineral and vitamins | 12 | 12 | 12 |
| Chemical composition, g/kg DM | | | |
| Ash | 60.6 | 97.5 | 103 |
| Protein[1] | 172 | 177 | 175 |
| Fat | 25.8 | 16.5 | 16.7 |
| Starch | 243 | 7.6 | 3.8 |
| Sugar | 34.2 | 241 | 238 |
| NDF | 318 | 280 | 277 |

W = diet with ground wheat; M = diet with sugar beet molasses; MBic = diet with sugar beet molasses and sodium bicarbonate.
[1] Feedstuff table: composition and feeding value of feedstuffs for cattle Report No. 91, Danish Agricultural Advisory Service, 2000, English version.

day. Measurements of $CH_4$ and $CO_2$ were taken in the metabolic cage continuously for 22 h for each animal, after which the heifers were moved to the respiration chamber for a similar time for the measurement of $CO_2$ emissions as described in the section below.

#### 2.2.2. Respiration chamber technique

The individual respiration measurements were performed for the measurement of total $CO_2$ in an open-air-circuit respiration chamber immediately after the metabolic cage measurements. Construction and function of the respirations chambers was described by Chwalibog et al. (2004). The animals had free access to the same diet in the chamber as it was in the metabolic cage and water was made available for 24 h. The climate in the chambers was kept constant at a temperature of 20 °C and a relative humidity of 60%. Chamber was calibrated by injecting know concentration of pure $CO_2$ and $N_2$ at the beginning of each measurement. The results obtained from calibrations indicate a high accuracy with an overall error of less than 1%. The concentrations of $O_2$, $CO_2$ and $CH_4$, temperature, relative humidity and rate of flow from the chamber were recorded automatically every 5 min. The exhaled $CO_2$ concentration was determined by the difference between the concentration of that in air-in and air-out. Data from the 22-h gas exchange measurements (for each diet) in the chamber was used as 2 h of the day were used to change animals.

### 2.3. Calculations

For the calculation of $CH_4$:$CO_2$ ratio from the breath samples, the average barn concentrations of $CO_2$ (705 ± 88.3 ppm) and $CH_4$ (26 ± 10.3 ppm) (means ± SD) were subtracted from the exhaled air concentrations to get the animal produced $CO_2$ and $CH_4$ concentrations. After correction, all values of corrected $CO_2$ below 400 ppm were removed in order to avoid the bias of samples containing a very low concentration of $CH_4$ and $CO_2$ generated when the animal's nose was not in the close proximity to the gas sampling inlet. The ratio of $CH_4$ to $CO_2$ was thereafter calculated.

Methane emission of the heifers was calculated from the breath sample analyses in 2 ways. Both calculations are based on the $CH_4$:$CO_2$ ratio measured in the metabolic cages and as described by Madsen et al. (2010), considering calculated total $CO_2$ calculated from heat production or measured total $CO_2$ in the respiration chamber ($CO_2R$). Heat production (HP) was calculated with animal parameters (metabolic weight, dairy weight gain, energy content of diet and days in pregnancy) as described in Eq. (1) by CIGR (2002). The amount of heat produced is necessary to know in order to calculate carbon dioxide production $CO_2$ (HP) according to the $CO_2T$ as described by Pedersen et al. (2008) and shown in Eq. (2). The value $CO_2$ (HP) was used in the $CO_2T$ calculated $CH_4$ production [Eq. (3)], and compared with calculated $CH_4$ produced based on $CO_2$ production measured in respiration chambers ($CO_2R$) [Eq. (4)]. The $CH_4$ (L/kg DMI) in the respiration chamber was calculated considering DMI from the previous day.

$$HP(watt) = 7.64 \times BW^{0.69} + Y\left[\frac{23}{M} - 1\right]\left[\frac{57.27 + 0.302 \times BW}{1 - 0.171Y}\right] + 1.6 \times 10^{-5} \times P^3 \tag{1}$$

$$CO_{2\ (HP)} = HPU \times 180(L) \times 24(h) \tag{2}$$

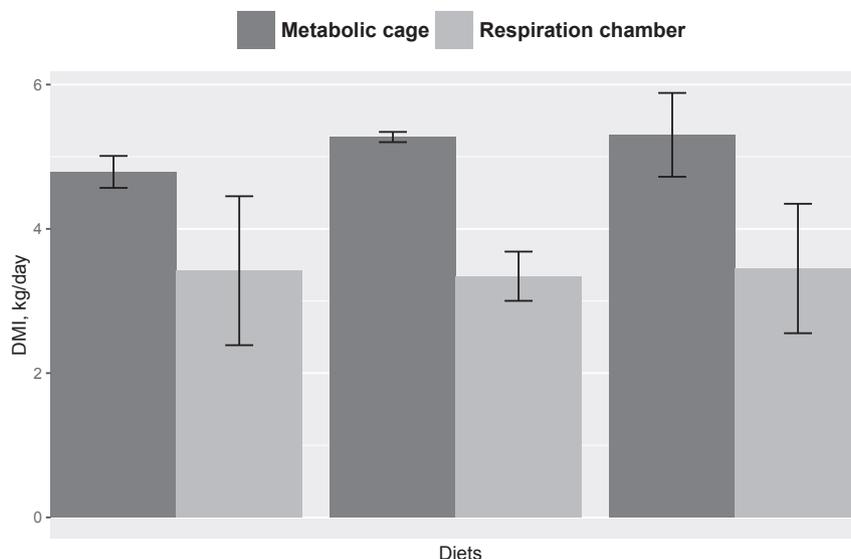$$CH_{4\ (HP)} = CO_{2(HP)} \times \frac{CH_4}{CO_2} \tag{3}$$

**Fig. 1.** Dry matter intake (DMI kg/day) of heifers fed 3 diets (W = wheat; M = molasses, and Mbic = molasses + sodium bicarbonate) in metabolic cage and respiration chamber. The bars indicate means ± SD of DMI (kg/day).

$$CH_{4(RC)} = CO_2R \times \frac{CH_4}{CO_2} \tag{4}$$

where HP = heat production of the animals; BW = body weight of the animals; $Y$ = daily weight gain set as 0.5 kg/day; $M$ = energy contents of the diet; $P$ = days of pregnancy of the heifers; $CO_{2\ (HP)}$ = carbon dioxide production (L/day) calculated based on heat production; $CO_2R$ = carbon dioxide production (L/day) measured in respiration chamber; HPU = heat producing unit calculated as HP/1000; $CH_{4\ (HP)}$ and $CH_{4\ (RC)}$ = methane calculated from $CO_{2\ (HP)}$ and $CO_{2\ (RC)}$; 180 = l of $CO_2$/HPU per hour; $CH_4/CO_2$ = measured $CH_4$:$CO_2$ ratio using the $CO_2T$ breath sample analysis.

### 2.4. Statistical analysis

All statistical analyses were undertaken in the R statistical program (R Development Core Team, 2013). Daily carbon dioxide emission and DMI during the period of time the heifers were in the metabolic cages and in the respiration chamber were first analyzed as a response variable with a linear model considering diet and heifer as fixed variables. Thereafter, the differences in average hourly methane breath concentrations during 24 h were tested with a linear mixed model using the lmer function from the lme4 package (Bates and Sarkar, 2009). The R package lmer Test was used to compute *P*-values directly from the model (Kuznetsova et al., 2012). The primary model was fitted by maximum likelihood for BW, diet (3 levels) and DMI as fixed variables and the heifer identification as a random variable. The final model in Eq. (5) was selected by the stepwise elimination of the non-significant variables. The estimates of the responses were produced by fitting the final model with Restricted Maximum Likelihood (REML). The model was validated using an analysis of variance (ANOVA) based on the Akaike Information Criterion. The model residuals were checked for normality and homoscedasticity by visual inspection of qq-plots.

$$y_{ij} = \mu + \alpha_i + X\beta_{ij} + \delta_j + \varepsilon_{ij} \tag{5}$$

where $y_{ij}$ is the response variable, $y$ = $CH_4$ in L/day and L/kg DMI of diet $i$ and heifer $j$, $\mu$ = overall mean, $\alpha_i$ = diet (W, M and Mbic), $X\beta_{ij}$ = DMI of heifer $j$ ($j$ is 1 to 3) for diet $i$, $\delta_j$ = random effect of heifer and $\varepsilon_{ij}$ is the model residuals.

## 3. Results

Dry matter intake (kg/day) in the metabolic cage was not different ($P > 0.1$) during the 3 measurement periods. Similarly, no difference of the DMI (kg/day) was observed in the respiration chamber during the measurement periods. However, the DMI (kg/day) was significantly higher ($P < 0.001$) in the metabolic cage compared with the intake in the chamber (Fig. 1). The $CH_4$ estimations for 2 methods are presented in Table 2. All 3 diets showed that daily $CH_4$ (L/kg DMI) emissions estimated by $CO_2T$ were of the same scale for the $CO_2R$. The measured $CO_2$ production in the respiration chamber (1,784 ± 193.5 L/day; means ± SD) was not different ($P = 0.39$) from the calculated $CO_2$ production (1,709 ± 52.1 L/day; means ± SD) using the $CO_2T$ method (Fig. 2). The calculated $CO_2$ (L/day) using the $CO_2T$ technique was positively correlated with the measured $CO_2$ (L/day) in the respiration chamber (Fig. 3) according to the body mass of the animal.

## 4. Discussion

### 4.1. Method comparison

The respiration chamber is the reference method for animal metabolism studies and total gas emissions, including $CH_4$. The $CO_2T$ is a newly developed technique which uses the $CH_4$:$CO_2$ ratio from breath sample analysis of the animals to calculate $CH_4$

**Table 2**
Methane production of heifers fed 3 different diets, estimated using different methods.

| Method | Diets | $CH_4$, L/day | $CH_4$, L/kg DMI |
|---|---|---|---|
| $CO_2T$ | W | 126.7[a] | 25.1[a] |
| | M | 144.8[b] | 28.2[b] |
| | Mbic | 154.0[c] | 30.2[c] |
| $CO_2R$ | W | 142.9[a] | 28.0[a] |
| | M | 148.6[a] | 29.0[a] |
| | Mbic | 151.5[b] | 29.8[b] |

$CH_4$ = methane; DMI = dry matter intake; $CO_2T$ = $CO_2$-method; $CO_2R$ = $CO_2$ measured in respiration chamber; W = wheat; M = molasses; Mbic = molasses + sodium bicarbonate.
[a,b,c] Values in the same column with different superscripts indicate differences ($P < 0.05$) between diets for each method.
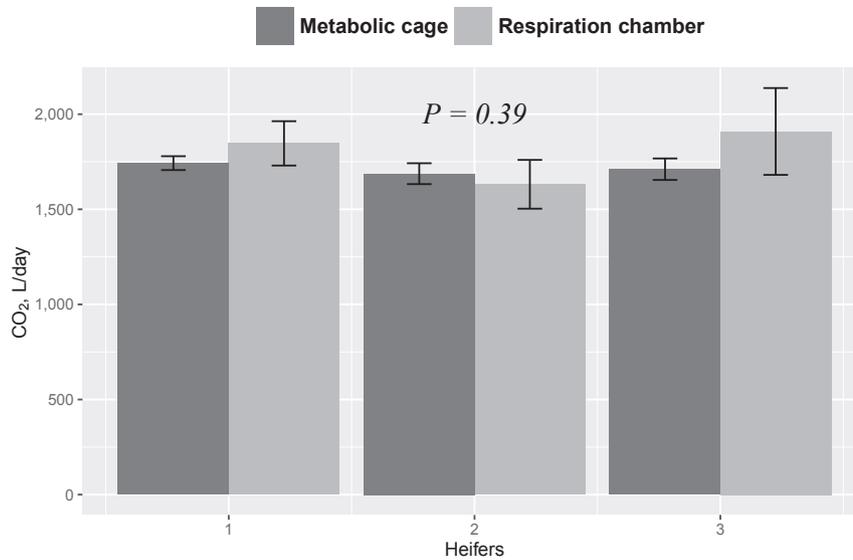
**Fig. 2.** Calculated total $CO_2$ (L/day) according to $CO_2T$ vs. measured $CO_2$ (L/day) by respiration chamber. The bars indicate means $\pm$ SD of $CO_2$ (L/day) production. The *P*-value is the model probability for significant difference of $CO_2$ (L/day) production between 2 measurement techniques.

production. The majority of $CH_4$ produced in the rumen is emitted through the eructation (Place and Mitloehner, 2010). The maximum $CH_4$ emitted from the hind gut of dairy cows is reported to be 13% of total daily methane emission (Ellis et al., 2008). Therefore, the $CO_2T$ method is valid in that the majority of the emission will be collected through breath sample analysis. The present results showed a lower DMI in the respiration chamber, in agreement with the previous study by Pinares-Patino and Clark (2008), who also reported lower intake in the respiration chamber. Dry matter intake has a large influence on the daily mean $CH_4$ emission (Boadi et al., 2004). Thorbek (1980) found that animals fed *ad libitum* in the barn showed a significantly lower intake when moved into the chamber. In the same study, animals had a higher intake in the respiration chamber when fed restricted in the barn. The DMI appears to be reduced in the traditional steel box respiration chamber which completely isolates the animals from others. Reduction of DMI may be less when dairy cows are placed in a modern designed plexiglass respiration chamber, as was done by Hellwing et al. (2012).

The $CH_4$ production per unit of DMI was comparable among all of the methods in the present study. The $CH_4$ (L/kg DMI) estimated by the $CO_2T$ was similar to the estimates by the $CO_2R$. This is presumed to be due to the fact that $CO_2$ produced in the chamber is not influenced by the one day lower DMI when in the chamber. The number of animals used in this study for the different methods was limited. Estimation of methane using the $CO_2T$ could be undertaken in a commercial farm situation where large number of animals could be considered and the animals have a more natural behavior (Haque et al., 2014a, 2015). A recent study indicated that the total
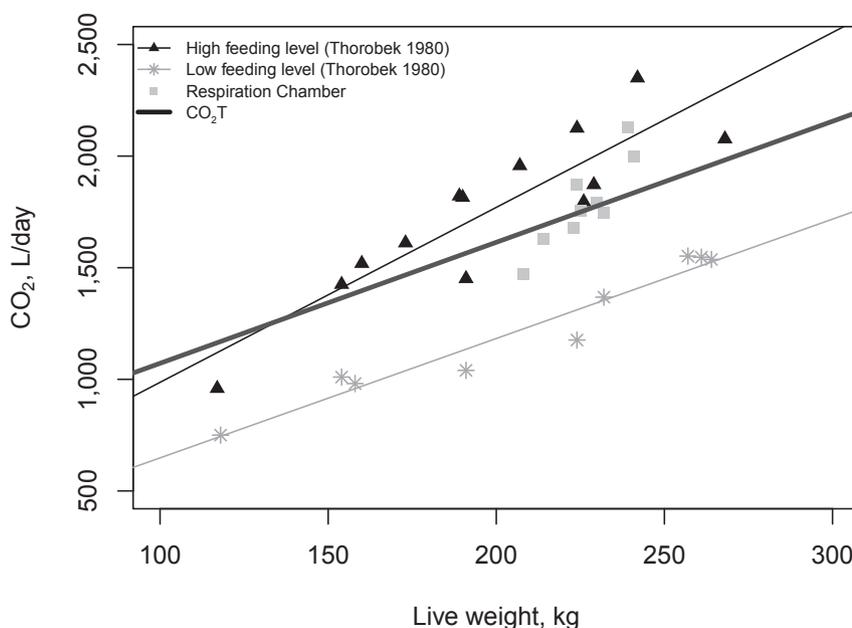


**Fig. 3.** Calculated and measured $CO_2$ (L/day) production from the heifers obtained by the $CO_2$ method ($CO_2T$) and respiration chamber ($CO_2R$) and compared with the previous results from respiration chamber study with growing bull calves at low and high feeding levels (Thorbek, 1980).

$CO_2$ concentration measured by the $CO_2T$ varies with variable muzzle movement, muzzle position and possible air mix or cross contamination (Huhtanen et al., 2015), which ultimately affects $CH_4$ estimation. In this study, cross contamination was avoided by specific data filtering system as described in section 2.3. Use of muzzle sensor in the sampling inlet would be a further development of the measurement of $CH_4$ and $CO_2$ concentration by the $CO_2T$. We assume that the precision of the methane $CO_2T$ estimates can be improved in this situation, either by measuring emissions from a large number of animals or measuring for a longer time without altering the natural movement of the animals.

## 4.2. Calculation of carbon dioxide production for methane estimation

The calculation of $CO_2$ production in the $CO_2T$ is based on the results from metabolism experiments reported in the last several decades. The total $CO_2$ production of animals can be calculated using body mass, growth and production information or using the nutrients intake and utilization. The $CO_2$ production of animals is determined by the type of diet and nutrient concentration, levels of intake and body activity, which is closely related to metabolism or heat production of animals (CIGR). The accuracy of $CH_4$ estimation using $CO_2T$ depends on the accuracy of calculated total $CO_2$ production (Madsen et al., 2014). The calculated $CO_2$ (by the $CO_2T$) and measured $CO_2$ in the respiration chamber ($CO_2R$) in this study showed a strong correlation ($r = 0.85$) with an average of 1,754 L/day and a deviation between the techniques of $\pm 53$ L/day. Moreover, Fig. 3 shows the $CO_2$ produced by bull calves fed either high or low feeding level (Thorbek, 1980)were respectively higher and lower than either the $CO_2T$ or $CO_2R$ estimations. Therefore, it can be concluded that the $CO_2T$ can predict the total $CO_2$ production with a reasonable accuracy because this prediction is comparable to the reference method i.e., respiration chambers. According to the $CO_2T$, $CO_2$ emission is multiplied with the $CH_4$:$CO_2$ ratio from breath sample analysis to calculate the daily $CH_4$ emission (Haque et al., 2014a, 2014b). The $CH_4$ estimation can therefore be influenced by the total $CO_2$ production as well as variation in the $CH_4$:$CO_2$ ratio (Haque et al., 2015). Bjerg et al. (2012) found diurnal variation of the $CH_4$:$CO_2$ ratio, which will influence the $CH_4$ estimation. This diurnal variation was considered in the present study by analyzing breath samples over 22 h. From the comparative values of $CH_4$ (L/kg DMI) estimated by the $CO_2T$, and $CO_2R$, it can be seen that the $CO_2T$ estimated $CH_4$ emissions with reasonable accuracy and precision.

## 5. Conclusions

The results show that the DMI was less in the respiration chamber than in the metabolic cages. All 3 diets showed a similar scale of methane estimation by the $CO_2T$ and $CO_2R$. The variation between estimated $CO_2$ productions was within the acceptable range for the 2 techniques ($CO_2T$ and $CO_2R$). The $CO_2T$ can predict $CH_4$ emissions with a reasonable accuracy and precision as compared with the chamber technique. The precision can be improved either by using more animals or longer measurement period.

## Acknowledgments

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