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Repeatability and variability of measurements of methane and carbon dioxide production in cattle housed in open-circuit respiration chambers

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Abstract. Gas recovery tests are necessary when the respiration chamber technique is employed for the measurement of greenhouse gases exhaled by domestic animals. A dataset of 98 individual measurements of methane and carbon dioxide production from cattle housed in two respirations chambers was used to assess variability and repeatability of the measurements performed. Analysis of variance was carried out to assess if statistically significant differences existed between chambers and between animals ($P < .0001$). Results showed the occurrence of a moderate but acceptable variability in methane production measurements between the chambers evaluated.

Key words: greenhouse gas, indirect calorimetry, uncertainty.

Repetibilidad y variabilidad de las mediciones de producción de metano y dióxido de carbono en bovinos alojados en cámaras de respiración de circuito abierto

Resumen. Se requiere de experimentos de recuperación de gases cuando la técnica de cámaras de respiración es empleada para la medición de gases de efecto invernadero exhalados por los animales domésticos. Una base de datos de 98 mediciones de metano (CH_4) entérico y dióxido de carbono producido por bovinos alojados en dos cámaras de respiración fueron usados para estimar la variabilidad y repetibilidad de las mediciones realizadas. Se realizó un análisis de varianza de los datos para evaluar si existían diferencias estadísticamente significativas entre las cámaras y entre los animales ($P < 0001$). Los resultados mostraron la ocurrencia de una variabilidad moderada, pero aceptable en las mediciones de producción de metano entre las cámaras de respiración evaluadas.

Palabras clave: gases de efecto invernadero, calorimetría indirecta, incertidumbre.

Repetibilidade e variabilidade de medições de produção de metano e dióxido de carbono em bovinos alojados em câmaras de respiração de circuito aberto

Resumo. Experimentos de recuperação de gás são necessários quando a técnica da câmara respiratória é utilizada para medição de gases de efeito estufa exalados por animais domésticos. Um banco de dados de 98 medições de metano entérico (CH_4) e dióxido de carbono produzido por bovinos alojados em duas câmaras respiratórias foi usado para estimar a variabilidade e repetibilidade das medições realizadas. Uma análise de variância dos dados foi realizada para avaliar se havia diferenças estatisticamente significativas entre as câmaras e entre os animais ($P < 0,0001$). Os resultados mostraram a ocorrência de variabilidade moderada, mas aceitável, nas medidas de produção de metano entre as câmaras respiratórias avaliadas.

Palavras-chave: gases de efeito estufa, calorimetria indireta, incerteza.

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Introducción

Greenhouse gas (GHG) emissions reached a record 51.5 gigatons of CO_2 equivalent ($GtCO_{2e}$) in 2019 excluding land-use change (LUC) emissions and 58.1 $GtCO_{2e}$ including LUC (United Nations Environmental Programme, 2021). The main GHGs that determine climate change are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Olivier, 2022), these gases contributed 73, 19 and 5 % of global total GHG emissions respectively, excluding land use, with F-gases accounting for the remaining 3 % (Olivier & Peters, 2020). Methane has a global warming potential of 28 to 36 times that of CO_2 over a 100-year period and 80 times that of CO_2 over a 20-year period (IPCC, 2021).

Livestock production contributes 14.5 to 19 % of global GHG emissions (Gerber *et al*., 2013; Johnson & Johnson, 1995). Enteric methane is a major source of greenhouse gas emissions from milk and beef production systems that contributes to global warming (Tricarico *et al*., 2022). Cattle are estimated to produce between 250 to 500 L of CH_4 per day (Johnson & Johnson, 1995) with up to 90 % of the CH_4 from ruminants is produced in the process of rumen microbial methanogenesis (McAllister *et al*., 2015).

Open-Circuit Respiration Chamber (OCRC) is the gold standard technique for measuring methane in ruminants provided that their gas recovery rates are close to 100 % (Garnsworthy *et al*., 2019). Charmley *et al*. (2016) conducted a meta-analysis of 1034 individual observations generated by experiments using OCRC and where forage-based diets (> 70 %) were used, records obtained from dairy cattle fed warm forages (220 records), beef-producing bovines fed with temperate forages (680 records) and meat-producing bovines fed with tropical forages (113 records). The authors reported CH_4 emissions g/d on the range, 237─623 (average 421 g CH_4/d) for dairy cattle in temperate regions, 78.9─241 (average 133 g CH_4/d) for beef cattle in temperate regions, and 32.2─184 (average 94.7 g CH_4/d) for cattle in tropical regions. Based on these results, they suggest using the value of 20.7 g CH_4/kg DM to estimate methane emissions in Australia.

In Mexico Ku-Vera *et al*. (2020) analyzed 125 individual methane yield (CH_4/kg DMI) data for *Bos taurus* × *Bos indicus* crosses that were fed low-quality tropical forages (> 70 %) and evaluated at OCRC, their results indicate a CH_4 production of 17 g/kg of DMI under those conditions. Which is comparable with the results presented by Charmley *et al*. (2016). However, although OCRC determinations are considered the gold standard technique to determine CH_4 emissions, it is necessary to make significant improvements that contribute to reducing variability and repeatability to improve the values obtained. This can be achieved by homogenizing the weight of the animals used, stabilize daily consumption and calibrate the OCRC on a routine basis (Fernández *et al*., 2019; Gerrits *et al*., 2018; Hristov *et al*., 2018).Dhumez *et al*. (2022) reported that determination of the gas recovery rate in respiration chamber facilities is a central prerequisite to assess the accuracy of methane emission quantification. However, data of recovery tests are seldom reported (Gerrits *et al*., 2017). Therefore, the objectives of this trial were to evaluate the inter-animal variability and repeatability of CH_4, and CO_2 production measurements carried out in crossbred (*Bos taurus* × *Bos indicus*) heifers housed in the open-circuit respiration chambers at the Laboratory of Climate Change and Livestock Production of the University of Yucatan, Mexico.

Materials and Methods

Ethical considerations

The experiment was approved by the Bioethics Committee and Manual for Research with Living Organisms and Environmental Conservation of the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Mexico.

Location

The experiment was carried out at the Laboratory of Climate Change and Livestock Production (LACCLIGA) of the Faculty of Veterinary Medicine and Animal Science of the University of Yucatan (21°15´N 83°32´W) in Mérida, México. The region has a warm sub-humid climate (Aw0) with rains in the summer. The average annual temperature is 26.8 °C and the average rainfall is 984.4 mm (García, 1981).

Animals

Six crossbred heifers (*Bos taurus* × *Bos indicus*) cannulated in the dorsal sac in the rumen (10 cm ─ Bar Diamond Inc.), 43 ± 4.4 months old and with an average body weight (BW) of 426 ± 56.1 kg, were used. The heifers were housed during the experimental period in individual metabolic crates equipped with feeders and drinkers; located in a roofed building, with a concrete floor and no walls. The heifers were dewormed and vitaminized 15 days before starting the experimental period. The dewormer used was an oral anthelmintic suspension (Oxfenil® Virbac México), 5 mL was administered for every 100 kg of BW (equivalent to 4.5 mg of oxfendazole/kg of BW). In addition, 5 mL of Vitafluid® Virbac México was administered individually intramuscularly (each mL contains = vitamin A, 500,000 IU; Vitamin D3, 50,000 IU; vitamin E, 50 IU).

Duration of the experimental period

The experimental period lasted 45 days, divided into five measurement subperiods. Each subperiod was nine days, during which heifers were interspersed in the two open-circuit respirations chambers (OCRC) for three days for determination of enteric methane emissions (one animal per chamber, starting on the day one to three with heifer one and two, ending each measurement subperiod with heifer five and six) and the other six days they remained in their respective metabolic crates.

Experimental ration

Chemical composition of the experimental ration is shown in Table 1, which consisted of 83 % Pennisetum purpureum (regrowth of 120 days) fresh and chopped (2.5 cm) and 17 % concentrate consisting of ground corn, soybean paste and a commercial mineral premix, covering the nutritional requirements for the maintenance of growing heifers (National Academies of Sciences & Medicine, 2021). The heifers had access to clean, fresh water always during the experiment.

Table 1. Proportion of ingredients in the ration and chemical composition.

Item	Treatment
Ingredients, g/kg of DM	
Pennisetum purpureum	830
Ground corn	73
Soybean meal	37
Minerals	60
Chemical composition, g/kg of DM	
Dry matter	942 ± 1.39
Organic matter	941 ± 1.22
Crude protein	52.7 ± 3.18
Neutral detergent fiber	709 ± 4.36
Acid detergent fiber	437 ± 13.88
Ether extract	8.13 ± 0.17
Ash	59.5 ± 1.22
Gross energy (MJ/kg DM)	15.4 ± 0.22

[1] Mineral premix contained (minimum values per kg) = 40 g of phosphorus, 120 g of calcium, 0.74 g of iron, 10 g of magnesium, 400 g of sodium chloride, 1.5 g of manganese, 1.5 g of zinc, 0.15 g of copper, 0.0018 g of iodine and 0.001 g of cobalt.

Experimental design

The experimental design used was completely randomized where the study factor was the chamber (levels = chamber 1 and 2), the number of replicates was six (randomly assigned, three to chamber 1 and three to chamber 2). The variables evaluated were dry matter intake (DMI), enteric methane (CH_4) production, and carbon dioxide (CO_2) production. These values were determined when the animals remained in the chamber.

Voluntary intake

Individual dry matter intake (DMI) was measured daily as the difference between the amount offered and that rejected the following day. The full ration was offered once a day at 8:00 h. The ration was adjusted every third day and a 10 % excess over the expected daily intake was offered. The rejects were withdrawn at 7:45 h. the following day. The samples of food offered and rejected for each day were kept at -4 °C until the end of the experiment for further analysis. The samples were dried in a forced-air oven at 60 °C for 72 h and then ground through a 2-mm mesh in a Wiley® mill (Arthur H. Thomas Co., Philadelphia, PA, USA). USA) and sent to the Animal Nutrition Laboratory the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Mexico for chemical analysis.

Chemical analysis

Dry matter content of ration and refusals were determined by drying sub-samples in a forced-air oven at 105 °C for 48 h (constant weight; # 7.007; AOAC International, 2016). Nitrogen concentration in the samples was analyzed (CP; N × 6.25) by the Dumas combustion procedure using a LECO CN-2000 3740 series equipment (LECO, Corporation, #2057; AOAC International, 2016). OM and ashes were determined by incineration in a muffle furnace at 550 °C for 6 h (AOAC International, 2016; # 923.03) and, the NDF content was determined using sodium sulfite without alpha amylase (Van Soest *et al*., 1991). Ether extract (EE) was obtained by the acid hydrolysis method using petroleum ether as solvent (#920.39; AOAC International, 2016)). GE concentration was determined in a calorimetric bomb (C200, IKA Works® Inc., Staufen, Germany).

Methane production

Measurements of CH_4 and CO_2 production were carried out in two open-circuit respiration chambers (OCRC) for periods of 23 hours. In total, 98 daily individual measurements of heat production in three heifers were analyzed. Heifers were fed a basal ration of chopped fresh *Pennisetum purpureum* grass and a supplement (ground corn + soybean meal); the level of feeding was slightly above maintenance. Construction, description, operation and calibration of the chambers is described in Canul-Solis *et al*. (2017) and Arceo-Castillo *et al*. (2019). The chambers (9.97 m^3 volume) were built from metal-sheet panels with double-layer insulation, equipped with concrete floor, internal cage of tubular steel, feeder, automatic waterer, and a lock for air intake. Acrylic windows (9 mm thick) were installed at both sides of the chambers so that cattle had visual contact between them in the adjacent chamber as well as with their surroundings. Chambers are equipped with air conditioning units to guarantee comfort [temperature: 23 ±1 °C and relative humidity (RH) = 55 ±10 %]. Chambers are fitted with a small fan to provide movement of air in the closed environment. To measure the concentration of CH_4 in air samples, an infrared analyzer (MA-10, Sable Systems International, Las Vegas, Nevada, USA) was used. The apparatus was calibrated before each run by zeroing it with pure N_2 (99.999 %; Praxair, Mexico) following the methodology described by Arceo-Castillo *et al*. (2019). Subsequently, a known concentration of CH_4 (1000 µmol/mol; Praxair® Gases Industrial Inc., Mexico) was released until the equipment stabilized at 0.1 ±0.03 and the measurements were then started. The respiration chambers had been previously calibrated by infusing a known amount of high purity methane CH4 (99.997 % purity) to assess recovery rates that ranged from 97-102 %, similar to those reported by Gardiner et al. (2015) and Machado *et al*. (2016). Carbon dioxide concentration in chamber air samples was determined with an infrared analyzer (Sable Systems, Las Vegas, Nevada, USA). The air inside the chambers was removed using two mass flow generators (Flow Kit 50-500; Sable Systems, Las Vegas, USA) at a rate of 1.0 L/min for each kg of animal live weight (Machado *et al*., 2016), generating an internal pressure of -276 Pa. The air samples passed through a drying column filled with Drierite (WA Hammond Drierite Company LTD®, USA) before being sent to the CH_4 analyzer through a multiplexer. The values obtained (µmol/mol) in the ExpeData® software (Sable Systems International®, USA) were extrapolated to 24 h.

Statistical analysis

To analyze the experimental data, the PROC GLM procedure of SAS 9.4 (SAS, 2012) was used. Mean separation was made by using the Tukey test with an alpha of 0.05. The data was analyzed under the following model:

$$Y_{ij} = \mu + \tau_i + A_{j(i)} + \varepsilon_{ij}$$

where:

Y_{ij} = response variable in question taken in the i—th chamber and the j—th cow.

μ = effect of the overall mean.

τ_i = It is the effect of the i—th camera.

$A_{j(i)}$ = Effect of the j—th cow within the i—th chamber.

ε_{ij} = is the experimental random error, $\varepsilon_{ij} \sim N(0, \delta^2)$.

Residual coefficient of variation (CV) and Repeatability (R) was determined according to those described by Huhtanen *et al*. (2013); Residual coefficient of variation was calculated as root mean square error divided by mean, and repeatability was calculated as R = δ^2_{Animal} + (δ^2_{Animal} + $\delta^2_{Residua}$l).

Results

Dry matter intake (DMI), CH_4 and CO_2 production are shown in Table 2. According to the analysis of variance performed on the data, DMI was statistically different between chambers (P=0.014) and between animals (P=0.007). Similarly, CH4 production (g/d and g/kg DMI), CO_2 production [g/d and g/kg DMI (methane yield)] and the CH_4/CO_2 ratio showed highly significant differences between chambers (P < .0001; Table 3), adjusted for the effect of animal.

On the other hand, the CV and residual CV for CH_4/d, CO_2/d, CO_2/DMI and CH_4/CO_2 ratio were more uniform in chamber 2 (Table 2), indicating less variability when we compared the values obtained with those of chamber 1. In addition, DMI and CH_4/DMI, the CV and residual CV were better in chamber 1. Intraclass correlation coefficient or repeatability for chambers 1 and 2, is in a range of 0.43 to 0.57 (Table 2); R values for DMI, CH_4/DMI, CO_2/DMI were higher in chamber 1, on the contrary, in chamber 2 CH_4/d, CO_2/d and CH_4/CO_2 presented the highest value of R.

Table 2. Mean values, variability and repeatability of feed intake and gas emissions.

	Item	DMI(kg/d)	CH_4(g/d)	CH_4/DMI(g/kg)	CO_2(g/d)	CO2/DMI(g/kg)	CH_4/CO_2(g/kg)
OCRC 1	Mean	9.26[a]	265[a]	29.1[a]	2205[a]	243[a]	8.30[a]
	CV	12.9	10.0	16.5	14.0	20.9	7.89
	Residual CV (%)	45.7	57.1	49.1	56.9	51.7	53.9
	Repeatability	0.47	0.52	0.43	0.51	0.44	0.53
OCRC 2	Mean	8.63[b]	320[b]	38.0[b]	2799[b]	332[b]	8.75[b]
	CV (%)	15.3	7.50	17.1	10.6	19.5	6.76
	Residual CV (%)	54.3	42.9	50.9	43.1	48.3	46.1
	Repeatability	0.53	0.48	0.57	0.49	0.56	0.47

OCRC = open-circuit respiration chambers; CV = coefficient of variation; DMI = dry matter intake; CH_4 = methane; CO_2 = carbon dioxide. Means with different letters denote significant difference at 5 % (Note Table 3).

Table 3. Statistical values for variation, F value and p-value between chambers and between animals.

Item		Variation	F-Value	p-Value
DMI	OCRC	8.85	6.27	0.014
	Animal (OCRC)	21.3	3.78	0.007
CH_4/day	OCRC	68078	333	<.0001
	Animal (OCRC)	38855	47.6	<.0001
CH_4/DMI	OCRC	1764	88.0	<.0001
	Animal (OCRC)	1180	14.7	<.0001
CO_2	OCRC	7945215	471	<.0001
	Animal (OCRC)	6657306	98.6	<.0001
CO_2/DMI	OCRC	180544	98.8	<.0001
	Animal (OCRC)	145744	20.0	<.0001
CO_2/CH_4	OCRC	4.49	16.5	<.0001
	Animal (OCRC)	11.5	10.6	<.0001

OCRC = Open-circuit respiration chamber; DMI = dry matter intake; CH_4 = methane; CO_2 = carbon dioxide.

On the other hand, the CV and residual CV for CH_4/d, CO_2/d, CO_2/DMI and CH_4/CO_2 ratio were more uniform in chamber 2 (Table 2), indicating less variability when we compared the values obtained with those of chamber 1. In addition, DMI and CH4/DMI, the CV and residual CV were better in chamber 1. Intraclass correlation coefficient or repeatability for chambers 1 and 2, is in a range of 0.43 to 0.57 (Table 2); R values for DMI, CH_4/DMI, CO_2/DMI were higher in chamber 1, on the contrary, in chamber 2 CH_4/d, CO_2/d and CH_4/CO_2 presented the highest value of R.

Discussion

Ruminants fed low-quality forages eructate considerable amounts of CH_4 gas to the atmosphere. Methane is a GHG which leads to a decrease in the energy available in feedstuffs for growth and milk production (Palangi and Macit, 2021), representing losses between 2 to 12 % of gross energy intake (Johnson and Johnson, 1995), which may otherwise be used for growth and production (Tapio *et al*., 2017). In the present work, the emissions of CH_4 represented on average losses of around 16.3 MJ/d of energy intake or its equivalent average Y_m = 11.9 %, which agrees with the report by Johnson & Johnson (1995). However, this value

Is above that reported by the Intergovernmental Panel on Climate Change (IPCC, 2019) of 7 % for cattle fed rations containing > 75 % forage and with digestibility ≤ 62 %. The average value of Y_m reported by Niu *et al*., (2018) in a meta-analysis of an intercontinental database is also lower than that found in the present trial (11.9 % vs. 6.0 %). $Y_{m's}$ higher to those usually reported (6.5 %-7.0 %) for grazing cattle are possible due to the fact that the herd in a given region or country has inconsistent production levels with the limits of feed quality as defined in the categories of IPCC (2019). Therefore, this organism recommends as a good practice a region or country-specific Ym, taking into account the quality of the diet offered to the animal as a validation method. In this respect, the quality of the diet (chemical composition and digestibility (Garnsworthy *et al*. 2019) will determine DMI (Congio *et al*., 2022), being these two factors important in the production of enteric CH_4 (Garnsworthy *et al*., 2019; Hristov *et al*. 2022). In the present work, average DMI was 8.95 kg and methane yield was 33.6 g/kg DMI. This high methane yield (g CH_4/kg DMI) may have resulted from the positive correlation existing between NDF content and methane production (Niu *et al*., 2018). This has been confirmed by Moraes *et al.* (2014) who reported that NDF may be utilized as an attribute of chemical composition of a ration to predict emissions of enteric methane. Recent results confirm this, as daily CH_4 emissions increased linearly ($p < 0.05$) from 325.2 to 391.9, from 261 to 399.8 and from 241.8 to 390.6 g CH_4/day in cows in the early stage, intermediate and late lactation, respectively, as NDF in the diet was increased (Dong *et al*., 2022). Nonetheless, the methane yield of 33.6 g CH_4/kg DMI in the present work is 67.2 % above the value reported by Niu *et al*. (2018) of 20.1 g CH_4/kg DMI. In order to explain this difference it is important to mention that NDF content of the ration employed in the present work was 200 % higher (709 ± 4.36 g/kg DM; Table 1) compared to the 354 g NDF/kg DM reported by Niu *et al*. (2018). The direct effect of a high content of NDF in the diet is to induce a decrease in DMI (National Academies of Sciences & Medicine, 2021) as a result of the filling effect of the undigested fiber residues in the rumen (Allen, 2000). Furthermore, retention time of digesta in the rumen is increased, as well as the time for fermentation as a result of the high NDF content and the lignin present in mature forages (National Academies of Sciences & Medicine, 2021) a situation which may have occurred with the forage used in the present study (120 days regrowth). The fibrolytic bacteria may be affected when the supply of protein is below the minimum 7 % for optimal rumen function (National Academies of Sciences and Medicine & Medicine, 2016) and therefore, limit fiber digestibility (Firkins, 2021). In this respect, apparent digestibility of NDF in the present work was 55.7 % on average (data not presented), and the supply of protein per kg DM was 5.27 % (Table 1), which is not enough for the appropriate function of fibrolytic bacteria in the rumen. Additionally, the amount of energy supplied to the rumen microorganisms is an important factor which affects the amount of nitrogen incorporated into microbial protein (Lu *et al*., 2019), in this study the levels of digestible and metabolisable energy estimated were 1.99 and 1.64 Mcal/kg DM (data not shown) which may have been a limiting factor for microbial growth. Thus, the associative effects of a high level of dietary NDF, a low supply of crude protein and energy in the diet, may have favoured a high rate of CH_4 production per kg dry matter intake, as microbial growth was restricted and the apparent digestibility decreased. Individual dry matter intake and methane production per kg dry matter intake can be observed in Figs. 1 and 2.

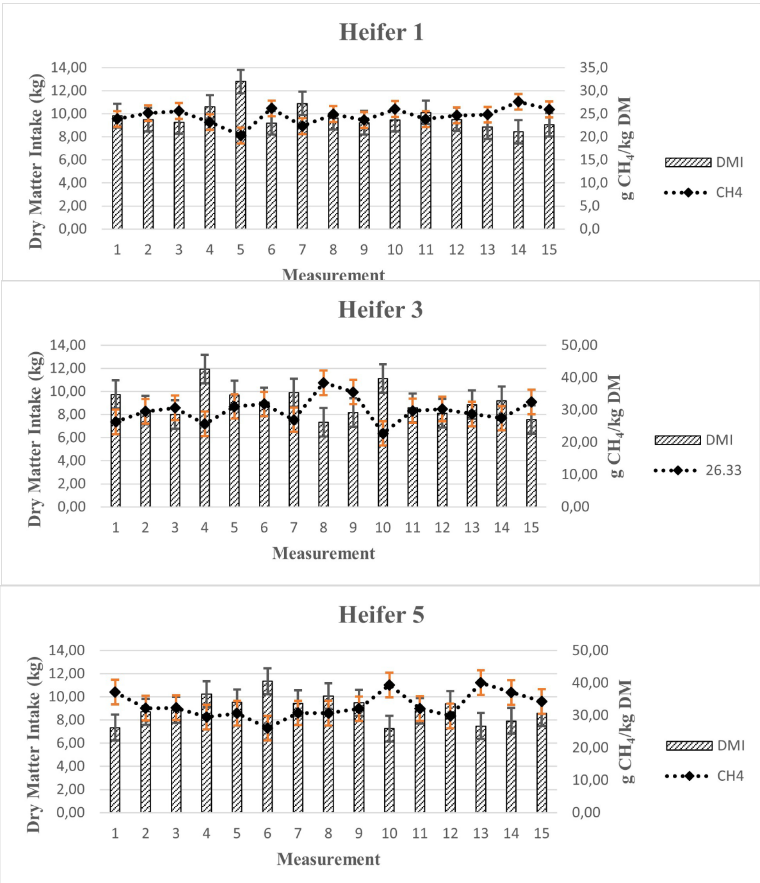

Figure 1. Emission of enteric CH_4 and DMI in heifers housed in Open Circuit Respiration Chamber 1.

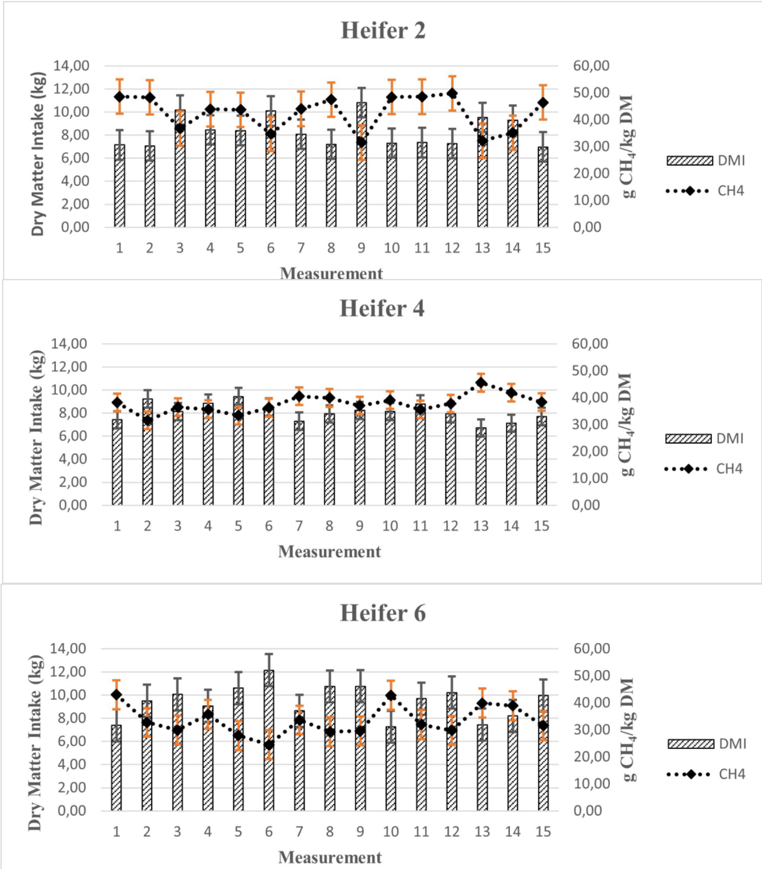

Figure 2. Emission of enteric CH_4 and DMI in heifers housed in Open Circuit Respiration Chamber 2.

Beauchemin *et al.* (2020) reported that methane production per day is a function of dry matter intake, chemical composition of the ration, rumen fermentability, genetics, physiology and the animal microbiome. Then, in order to understand variability in CH_4 production (Beauchemin *et al.*, 2022) between animals so as to evaluate with precision phenotypes low in methane, is a useful tool which will allow selection the animals more efficient in the use of nutrients. The average coefficients of variation (CV) estimated for the emission of methane and DMI en the present work were 11.1 and 7.2, 16.4 and 15.1, 14.4 and 13.2, 9.6 and 9, 13.2 and 12.2, 14.8 and 17.4, for heifers 1, 2, 3, 4, 5 and 6, respectively. This allows a individual classification of the heifers according to the highest and lowest levels of methane emission and DMI in the following order 4>1>5>3>6>2. As it can be observed in Figures 1 and 2, variability between animals for CH_4/day and CH_4/kg DM can be attributed to dry matter intake itself, to the nutrient content of the experimental ration (Table 1) (Huhtanen *et al.* (2013) and to body weight of heifers (Hristov *et al.*, 2017), which agrees with Huhtanen *et al.* (2013). However, a certain percentage of the values may be explained also by the variability associated to the measurement method itself (Hristov *et al.* 2018).

In the present work CV and residual CV presented values more steady in chamber 2 for methane production per day. However, when the methane yield data were adjusted to the effect of diet and that of DM intake, chamber 1 results were more uniform. This trend is also observed in the work carried out by Huhtanen et al. (2013). Hristov et al. (2018) pointed out that the CV for average methane emission rate per day was on average 30 % for systems using OCRC. However, they also express a low variability as that recorded in this trial (chamber 1 = 10 % vs chamber 2 = 7.5 %) which not always means a highly precise measurement, making it clear that each method must be evaluated by the researchers who in the light of their experience and with the data available, may determine if their particular method can be used with confidence for methane measurements for the conditions and specific objectives of their experiment and animals used (Hristov *et al.*, 2018). On the other hand, CV and residual CV for the relationships CO_2/d, CO_2/DMI and CH_4/CO_2 were lower in chamber 2, which allows confidence in the results obtained. Repeatability determined between respiration chambers was more consistent for chamber 1 for the expressions of CH_4/d, CO_2/d and the relationship CH_4/CO_2, while chamber 2 showed repeatability values consistent for emissions of CH_4 and CO_2 per kg DMI. Wang *et al.* (2020) pointed out that repeatability of data may be defined as the consistency between repeated measurements resulting from the same measurement technique. This methodology is utilized to define the amount of variation in the measurement data of an open-circuit calorimetry system, since the variation in measurements is compared with the total variability observed, and, as consequence, it defines the capacity of the measurement system (Fernández *et al.*, 2019). The coefficient is very good when the value is 1. However, according to Martin and Bateson (1986; 2021) values of repeatability obtained for chambers 1 and 2 are within the range 0.43 to 0.57, which suggest a moderate repeatability (R between 0.4 and 0.7; Martin and Bateson, 1986; 2021) and it is acceptable for experiments with animals as long as the results are statistically significant as it occurred in the experiment hereby described (see Table 3). Experiments with sheep housed in respiration chambers have reported repeatability in methane measurement of 79 % (Robinson *et al.*, 2014) and 76 % (Robinson *et al.*, 2016), respectively. Pinares-Patiño *et al*. (2013), Oddy *et al.* (2018) and Fernández *et al*. (2019) reported repeatability values of 89, 65 and 79 % for measurement of methane. Repeatability in the present trial for chambers 1 and 2 for methane emission per day and for methane produced per kg dry matter intake remained in an acceptable range. Nonetheless, it is necessary to reach significant improvements in future work, by using cattle with an homogenous live-weight, keep dry matter intake in a stable pattern per day, check routinely the chambers for leaks and demonstrate rates of methane recovery of around 100% (Fernández *et al.*, 2019; Gerrits *et al.*, 2018; Hristov *et al.*, 2018; Oddy *et al.*, 2018; Pinares-Patiño *et al.*, 2013; Robinson *et al.*, 2014, 2016).

Conclusions

Based on the results hereby presented it can be concluded that there is a moderate but acceptable variability in the measurements of methane production of cattle housed in open-circuit respiration chambers. It is important to carry out frequent checks of cracks in the seal of the chamber doors and windows, as well as assess the uncertainties (instrumental noise) in the air sampling duct along with flow measurements.

Conflicts of interest: The authors declare no conflict of interest.

Ethics statement: Heifer were handled in accordance with the ethical standards and technical specifications for the production, care and use of laboratory animals enforced at the Faculty of Veterinary Medicine and Animal Science (FMVZ; CB-CCBA-D-2021-001) of the University of Yucatan (UADY), Merida, Mexico, following the NOM-062-ZOO 1999.

Author contributions

Ever del J. Flores-Santiago: Conceptualization, Resources, Writing. Validation. Formal analysis. Investigation. Methodology. Visualization, Project administration. Writing – original draft. **Humberto Vaquera-Huerta:** Formal analysis. **Jesús Miguel Calzada Marín**: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **Jesús Miguel Calzada Marín:** Methodology. Methodology. Validation. Investigation. **Juan C. Ku-Vera**: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. Formal análisis. Methodology. Writing – original draft. **Paulina Vásquez Mendoza:** Investigation. Writing – original draft. review & editing, Supervision, Funding acquisition. **Roberto González-Garduño:** Formal analysis. Methodology.

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