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Design and operation of a spirometry mask to quantify exhaled methane emission by grazing cattle

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Abstract

To have an alternative technique that allows the quantification of methane emissions in cattle under grazing conditions continuously and individually without significantly affecting the behavior of the animals, an equipment consisting of a system for the measurement of total air exhaled and another one to take and store samples of exhaled air was designed. The prototype obtained and put into operation was manufactured with low-cost and easily available parts. The experimental cows easily adapted to the equipment in such a way that in a few hours they moved through the pasture without showing signs of stress, grazed calmly, consumed water and the food supplement, chewed the cud, lay down and got up without any apparent difficulty. In this way, the equipment satisfactorily fulfilled the objective for which it was designed. Due to its versatility, the equipment can be used in studies that require the measurement of lung function and respiratory rate of cattle under grazing.

Key words: *exhaled air, respiratory function, respiratory rate*

Introduction

Methane (CH4) is a gas that significantly contributes to the greenhouse effect, a natural atmospheric phenomenon in which gases like this one trap infrared radiation, increasing the temperature of the earth's surface to levels compatible with life (IPCC 2021). However, the increase in greenhouse gas emissions into the atmosphere due to anthropogenic activities such as livestock farming has led to an abnormal increase in global temperature (Bačėninaitė et al 2022). Quantifying methane emissions in cattle, then, is essential to carry out studies that allow to evaluate the effect that various factors have on these emissions and, with this, establishing guidelines that allow them to be reduced. Currently the only method that allows continuous quantification of methane emissions in individual animals under grazing conditions is sulfur hexafluoride (SF6) (Thompson y Rowntree 2020, Moate et al 2021). The method is based on the use of SF6 as an indicator of methane emissions under the assumption that its emission rate is like that of methane (Johnson et al 1994). The implementation of the method, however, is costly, complex, and laborious (ICAR 2020) to the point that it has been necessary to publish several manuals that guide researchers in assembling the method (INIA 2015, Berndt et al 2014, Jonker and Waghorn 2020). Among the main limitations are the fact that it requires the manufacture of special SF6 permeation capsules and the calibration of release rate takes several weeks in laboratory; however, between 6 to11% lower release rates in tubes placed in rumen fluid than in laboratory has been recorded (Pinares et al 2008, Deepa et al 2016); likewise, it has been established that the release rate of SF6 is relatively constant while that of methane in the rumen varies throughout the day (Pinares et al 2008); and that the SF6 is a gas with a potential 23500 times greater than CO2 with a half-life in the atmosphere of more than 3200 years (EPA 2023). On the other hand, steel tubes must be manufactured on which a vacuum must be generated and a system for taking air samples must be assembled, which, likewise, must be calibrated and requires specialized supplies (Berndt et al 2014). Considering the above, there is a necessity to develop methods that can overcome the challenges posed by SF6 in measuring enteric methane emitted by grazing cattle. It is within this context that the spirometry mask described here was designed.

Spirometry is a test used to evaluate lung function and is based on the measurement of the amount and speed of exhaled and inhaled air (Ambhore et al 2023). The first equipment designed to carry out spirometric studies dates from the end of the 18th century (Davy 1800). However, the first spirometric masks were designed in the mid-19th century to use in humans. Andral and Gavarret (1843) used a mask made of waterproof copper that covered the entire face of the patient and was provided with a glass while the edges were covered with a rubber cord to protect the skin and prevent the loss of exhaled gas. Spirometric masks for animals were already used at the beginning of the 20th century and, unlike Andral and Gavarret (1843), they did not cover the face as they breathed in directly from the nostrils (Tissot 1904). The design of this mask, however, prevented the patient from consuming food or water as it stretched over the mouth. Warren and Brody (1932), for their part, used spirometric masks for their indirect calorimetry studies with bovines that completely covered the animal's muzzle, thus preventing them from consuming water and food. These types of masks are still used today (Maia et al 2014, Camerro et al 2016, Tedeschi et al 2022) which is why they significantly limit the behavior of the animals and have a limited use for animals in the stable to carry out periodic measurements.

The objective of the spirometric mask designed for this work was to measure the entirety of the air exhaled by the animals without affecting their behavior under grazing conditions. Additionally, it aimed to collect and store samples of exhaled air to determine the content of gases that, specifically methane in this case. To achieve this, various electronic and mechanic components was combined as a “technological chimera”.

Measurement of exhaled airflow

From an oval container made of high-density polyethylene (HDPE) with a capacity of 2L (Figure 1A), a handmade spirometric mask was designed in such a way that it adjusted to the size and shape of the nose of the animals (Figure 1B). On the edge of the mask, a flexible rubber gasket was inserted and adhered with plastic silicone to prevent lacerations on the animal's skin and create an airtight seal that prevents the loss of exhaled gases (Figure 1C). In the middle part of the upper surface of the mask, a flexible corrugated tube of 1” and 20 cm in length was inserted, which was deformed with heat to fit ergonomically to the shape of the animal's face (Figure 1C). This tube was firmly adhered to the mask by heat silicone both externally and internally. At the other end of the corrugated tube, a smooth 1” PVC joint was inserted with a reduction of 1” to ¾” that was adhered with liquid silicone (Figure 1C). A ¾” digital flow sensor (model FS300A G3/4”) was inserted into the free end of the PVC union using a 3.0 cm union of clear PVC hose in such a way that it will fit tight (Figure 1D). The flow sensor was connected to a 5V LED reader through a quick electrical connection that reads the total volume of exhaled air during the time it remains connected. The LED reader was connected to a 5V and 5000mAh portable battery (King Power, model KP-56). The entire system was then installed on a 1” riata head-harness using two plastic ties. These plastic ties were inserted into the lateral wings of the mask to securely fasten it to the right and left rings of the harness, preventing any displacement of the mask.

Flow Sensor Calibration

To calibrate the flow sensor, a ¾" hose was inserted at the outlet of the digital flow sensor. This hose was then connected to a plastic bag with an 8L capacity, which was used to store all the air from six independent exhalations in each one of four experimental cows. Simultaneously, the volume reading determined by the sensor was recorded on the display. To determine the air stored after each exhalation, the bags were hermetically closed and submerged in a 20L volumetric container filled with 7L of water. A flat lid was used to completely submerge the bag in the water. The volume of displaced water corresponded to the volume of exhaled air. From the readings of the real volume and the volume determined by the sensor, the relationship between these readings was established. This relationship was then used to modify the "K" factor of the display, according to the manufacturer's instructions.

Collection and storage of exhaled air samples

For the taking and storage of exhaled air samples, a second system was designed. This system involves installing a 1/8" "L" coupling in the middle part of the corrugated tube (Figure 2A). From this coupling, a silicone hose of approximately 60 cm is connected to a 5V peristaltic pump (Model 500 Motor Pump – Type C) (Figure 2B). Another silicone hose of approximately 40 cm emerges from this pump and is inserted into two aluminum-based gas collection bags with a capacity of 5L (Ningbo Hongpu Experimental Technology Co, Ltda., Zhejiang, China). These bags are attached to a 1” riata strap tied around the thorax of the experimental animals (Figures 2C and 2D). The collected air samples will later be analyzed for their methane content using a portable analyzer with an N55A 22YS catalytic combustion sensor (Nemoto Special Chemicals Co., Ltd., Japan).

To control the gas sampling with the peristaltic pump, a 5V timer (Aideepen ELEC&Lifes, China) was incorporated into the system. The timer activates the peristaltic pump for 30 seconds every 90 seconds (Figure 2B). During the 30 seconds that the peristaltic pump remains activated, a handmade electromechanical sensor detects when the animal exhaled air and activated the system but turned it off when it inhaled air (Figure 3). This sensor ensures that the stored air samples corresponded exclusively to exhaled air. Then peristaltic pump and timer are connected to 5V 5000mAh portable batteries.

Measurement of respiratory rate

From the electromechanical sensor, a digital craft counter was connected, which was manufactured from a 1.5V CASIO HL-815L Calculator. The positive (+) and negative (-) poles of the result key (“=”) were connected to two wires that were removed from inside the calculator and installed in the electromechanical sensor (Figure 4). As a result, when the animal produced an inhalation, contact was made between the two cables of the “=” key, resulting in the addition of that inhalation. When the animal exhaled, the contact was interrupted, and the peristaltic pump was activated. In this manner, the counter added the number of inhalations while the system remained activated.

To activate the counter, it was turned on, the "1 + 1" keys were activated, and it was connected to the electromechanical sensor using a fast connection to initiate the inhalation count. The respiratory rate was calculated by dividing the final sum of inhalations by the duration of the counter´s activity. Since the number of inhalations is equal to the number of exhalations, dividing the total volume of air exhaled by the final sum of inhalations provided the volume of air exhaled for each exhalation.

The equipment can be integrated with different sensors, which increase the number of variables that can be measured depending on the is intended study. Additionally, it allows for the incorporation of a Bluetooth module to transmit the information directly to a computer.

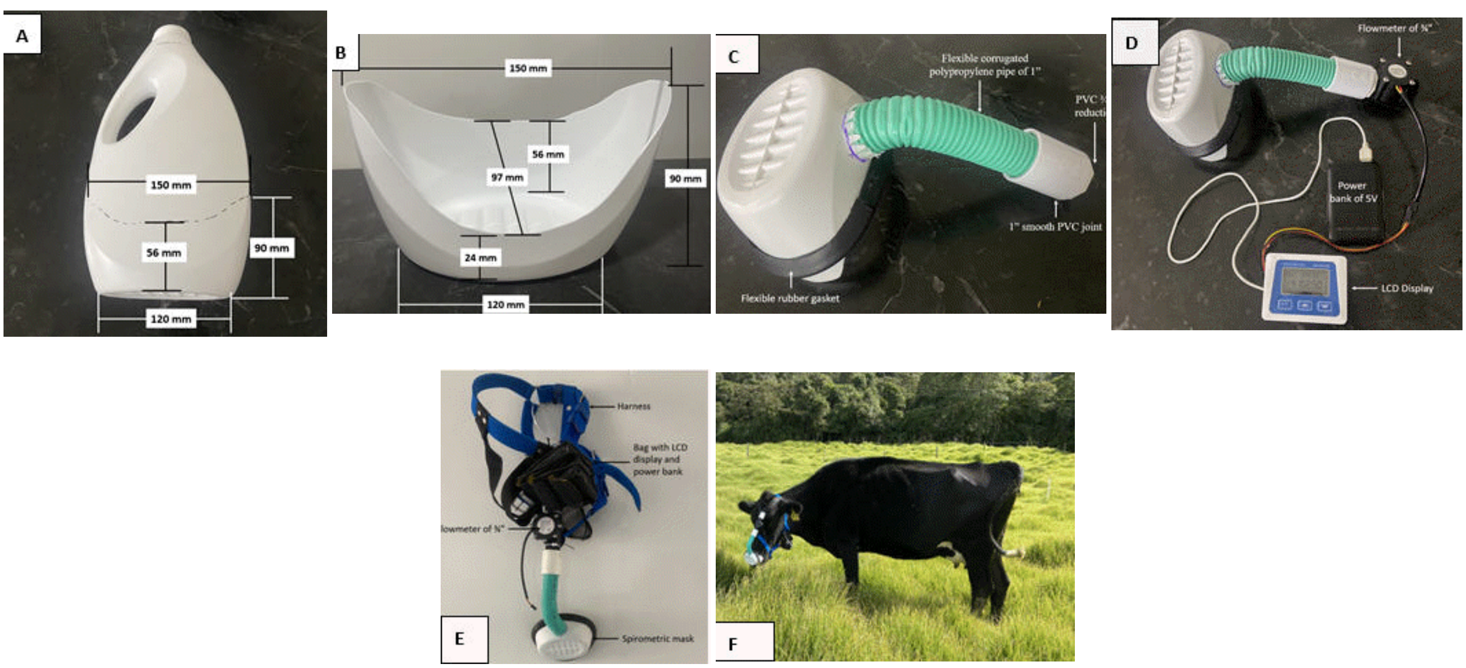

Figure 1. Manufacturing process and assembly of the spirometric mask. The description of each image is in the text

Calculation of methane emission

The amount of methane emitted by the animal through the exhaled air during the time that the equipment remained activated was calculated by multiplying the total volume of exhaled air during the measurement time (expressed in L/min) by the methane concentration (ppm) that was determined in the air sample stored in the same period. To convert from ppm to L/min, the following calculation was performed:

CH4, L/min = Ve*CH4c/1000000,

where **Ve** is the volume of exhaled air (L/min) and **CH4c** is the methane concentration (ppm). To express the CH4 emission in g/min, the value obtained in L/min was multiplied by 0.716 (Marumo et al 2023).

Evaluation of equipment

The evaluation of the equipment´s operation was conducted using cows from the dairy program of the Paysandú Experimental Station of the Universidad Nacional de Colombia, Medellín headquarters, located in the Corregimiento of Santa Helena, at an elevation of 2200 meters above sea level. The average temperature of it is 16^{o}C, with an average relative humidity of 75%. The protocol for the use of masks with these animals was approved by the Animal Research Ethics Committee of the Universidad Nacional de Colombia, Medellín headquarters (CICUA-19-23).

The equipment weight approximately 1.4 kg, which is less than half the weight of SF6 equipment (Pereira et al 2021). It was assembled on four cows, including two Holsteins, one F1 Holstein x Black-eared White cross, and one Jersey cow. Two cows were lactating and two others was dry. These cows had never worn a head-harness. The assembly procedure was quick, taking no more than 10 minutes. Prior to equipment assembly, cows were restrained by the neck to a firm post to minimize head movement while the equipment was being assembled. Subsequently, the electrical systems and hoses for taking and storing exhaled air samples were connected.

The four cows wearing the spirometric masks grazed on Kikuyu grass (*Cenchrus clandestinus*) meadows for approximately five to six continuous hours, including the time required for the milking process, which lasted about half an hour.

The animals quickly adapted to the equipment, as within two hours they resumed their normal grazing activities, such as moving around the meadow, intake the grass, consuming water, and food supplements, lying down, and getting up, without showing any apparent difficulty. Initially, the animals remained still for at least 30 minutes and then began to walk slowly. As these animals were in the presence of other lactating cows at the Experiment Station, many of them looked curiously at the cows wearing the equipment, approached them, sniffed at them, and attempted to lick the equipment. In response, the experimental cows sought to distance themselves from the others. This process took at least two hours before the other cows calmed down and stopped bothering the experimental ones.

Both lactating and dry cows were brought to the milking barn to evaluate its behavior. During the milking process, the cows showed no signs of restlessness or discomfort with the equipment. They consumed the offered food supplement without difficulty. Other authors have reported that equipment installed on the head of cattle, such of the SF6 equipment (Pereira et al 2021), the helmet constructed by Yousef and Johnson (1965), the facial mask developed by Maia et al (2014) for indirect calorimetric studies, and the device that simulate methane capture equipment located in the frontal part of the head of dairy designed by Damian et al (2022), neither significatively affect the behavior of experimental cattle.

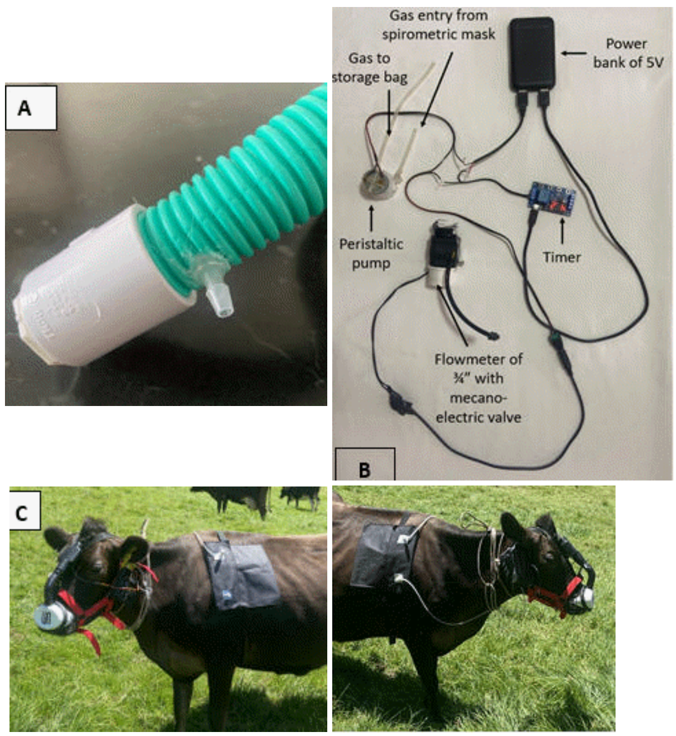

Figure 2. Assembly of the system for taking and storing exhaled air samples. The description of each image is in the text

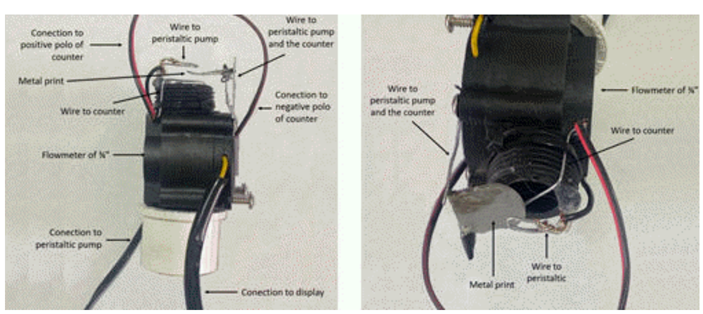

Figure 3. Detailed view of the electromechanical sensor

Figure 4. Connection points of the positive ("+") and negative ("-") poles of the CASIO HL-815L Calculator used to count inhalations

Table 1 presents the results of the calculation of CH4 emission of four cows under grazing conditions while wearing the spirometric mask. The volume of air exhaled per minute (tidal volume) was similar to the values reported by Gallivan et al (1989a), Gallivan et al (1991), and Dißmann et al (2023) for cows with comparable weight, but higher than the volume reported by Reeves et al (1962) and Gallivan et al (1989b). The respiratory frequency was within normal range, oscillating between values reported by others (Reeves et al 1962, Gallivan et al 1989a 1989b, Dißmann et al 2023).

The CH4 concentrations in the exhaled air samples fell within the range reported by Blaise et al (2018), Negussie et al (2017) and Huhtanen et al (2015) using different models of infrared sensors, and as the reported by Rey et al (2019) utilizing a laser methane sensor. The CH4 emission values found in this study were in between the values reported by Blaise et al (2018) using an equipment installed on the face of cows and sampling air directly from the nostrils, but approximately half of the values reported by Noguera y Posada (2017) in cows fed a diet based on *Cenchrus clandestinus* grass in a respirometric chamber, and by Arias et al (2023) in cows also fed a diet based on *Cenchrus clandestinus* grass but with a polytunnel technique. These differences can be attributed to the fact that the respirometric chamber and polytunnel methods calculated CH4 emissions including oral, nasal, and anal emissions, whereas the spirometric mask use in this experiment measures only the nasal emissions resulting from pulmonary exhalation.

The excretion of enteric CH4 in ruminants in complex. Approximately 77% to 90% of enteric CH4 is produced in the rumen, while 10% to 23% is produced in the hindgut (Dini et al 2012, Murray et al 1976). However, less of 5% of CH4 produced by ruminants in large intestine is excreted through flatus. This is due to the high absorption of hindgut CH4 and its transportation through blood to lungs (Murray et al 1976). On other hand, CH4 produced in rumen is primarily expelled through eructation via the esophagus, whereas nearly 90% of CH4 produced in large intestine can be release through the breath (Murray et al 1999). A portion of the CH4 produced in the rumen is also absorbed and transported to the lungs through the blood, then released through respiration (Heywood and Wood 1985). Belching is a complex process in ruminants, in which the nasopharyngeal sphincter directs a significant portion of the belched gas from the rumen through the esophagus into the trachea and lungs before being expelled with exhaled air (Reece and Rowe 2017). Therefore, CH4 produced from the hindgut and rumen is eliminated through breath, either directly or via transportation through blood.

The respiratory rate is higher than eructation rate (Mortola and Lanthier 2005), and variation in these rates occur due to factor such environmental conditions, time after food intake, and type of food (Pickering et al 2015). Thus, to minimize variation in exhaled CH4 emission values, it is important to take air samples over several hours and gather information about respiratory rate and volume of exhaled air. It is estimated that over half of the eructed gas is eliminated through noises (Reece and Rowe 2017). Therefore, the CH4 emissions reported in this study via exhalation reflect the complex process of CH4 elimination in ruminants through this route, which would represent approximately half of the total CH4 emitted by ruminants.

Table 1. Characteristics of experimental cows, of respiratory function and CH4 concentration and exhalated emission.

Cow	Weight, kg	Production, L/d	AE, L/min	RF, E/min	CH4, ppm	CH4, L/d	CH4, g/d
1	482	-	4,93	26,5	525	98,7	70,7
2	598	12,0	3,78	26,8	970	144	103
3	647	-	5,02	25,3	1753	307	219
4	595	11,0	4,27	27,6	1047	175	125
Mean	580		4,50	26,6	1074	181	130
SD	69,9		0,590	0,951	507	89,4	64,0
CV	12,0		13,0	3,59	47,3	49,3	49,3

AE: air exhalate; RF: respiration frequency; SD: standard deviation; CV: coefficient of variation

A preliminary analysis of regression of the dates recorded in this study show a clear relationship between the live weight of experimental cows and methane emission (r2 = 0.978, *p*<0.001). This can be due that heavier animals have a higher feed intake and consequently, higher CH4 production (Hardan et al 2022; Hristov et al 2018). This relationship, however, has not been so clear in other works. In this regard, Hristov and Melgar (2020) points out that this depends on the technique used to determine methane emissions. In general, this relationship is high for respiration chamber data, but low for GreenFeed system data (Niu et al 2018) and intermediate with SF6 (Arbre et al 2016, Pires et al 2018). The analysis of regression of this study suggests that the spirometric mask can be a good method to quantification CH4 emissions in cattle under grazing conditions.

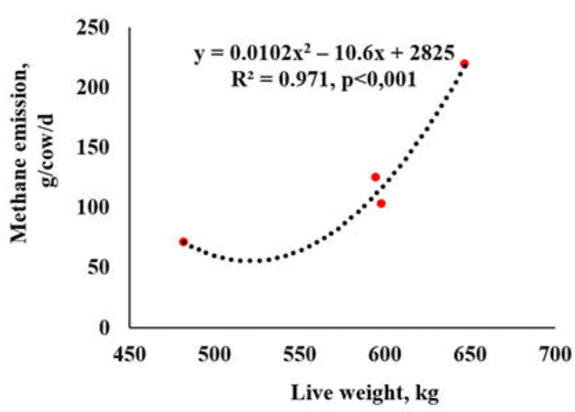

Figure 5. Relationship between the live weight of experimental cows and methane emission

It can be concluded that the spirometric mask designed and evaluated in this study is a low-cost equipment that is easy of manufacture and adaptable to experimental animals. The CH4 emissions calculated whit the equipment is consistent with the expected quantity of this gas exhalated through this route.

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