Received: 23 March 2022 | Accepted: 2 September 2022

DOI: 10.1111/jpn.13780

SHORT COMMUNICATION

Ruminant

Journal of Animal Physiology and Animal Nutrition WILEY

A new equipment for continuous measurement of methane production in a batch in vitro rumen system

Matteo Braidot | Chiara Sarnataro | Alberto Romanzin | Mauro Spanghero

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy

Correspondence
Matteo Braidot, Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio, 2/A, 33100 Udine, Italy.
Email: braidot.matteo@spes.uniud.it

Abstract

A new rumen batch fermentation system that allows continuous measures of total gas (GP) and methane production (MP) was tested. The fermentation system is composed of glass bottles connected to gas counters (Ritter Apparatebau GmbH & Co. KG) and an infrared gas analyser that measures the methane concentration. The system allows direct and continuous measurement of GP and MP for accurate kinetic studies. The aim of the work was to test the rumen fermentation system and compare the GP and MP kinetics obtained. Barley meal (BM), alfalfa hay (AH), corn silage (CS), and soya bean hulls (SH) were used as substrates in four consecutive fermentation runs. Cumulative volumes of GP and MP and the percentage of methane on total GP were recorded continuously until 48 h and average values at 1 h intervals were fitted with an exponential model with a lag phase reaching a good fit ($R^2 > 0.992$). GP and MP reached the highest plateau levels for SH (1836 and 370 ml, respectively; $p < 0.01$) and the lowest for AH (1000 and 233 ml, respectively). The remaining substrates showed intermediate values. MP kinetics showed a discrete lag phase (from 0.09 to 1.12 h), whereas it was equal to zero for the total GP (except for SH). The methane concentration in gas flowing increased rapidly at the beginning of fermentation (from 0.35 to 0.95 h^{-1}) and reached a plateau after approximately 8–12 h. In conclusion, the rumen fermentation system evaluated generates methane data comparable to those reported in the literature and allows simple continuous measurement of methane release throughout fermentation.

KEYWORDS

Gas production, In vitro fermentation, Methane, Rumen batch systems

1 | INTRODUCTION

Emissions of greenhouse gases, such as carbon dioxide and methane, into the atmosphere, cause global warming and serious damage to the planet's ecosystem. The livestock sector is responsible for 14.5% of anthropogenic greenhouse gas emissions, according to the Food and Agriculture Organization of the United Nation. Of these, ruminant enteric methane production account for 40% (Gerber et al., 2013). The EU recently agreed to reduce 36% of methane emissions by 2030 compared to 2005 levels (Commission of the European Community, 2020), and as a result, new feeding strategies to

reduce rumen methane emission in livestock systems are a growing research topic.

Given the limitations of animal experiments, due to costs, time, ethical concerns, and standardization constraints, there is an increasing appreciation for experiments that do not involve animals, such as in vitro rumen fermentation experiments. The developed in vitro batch systems based on gas production (GP) and adapted to methane measurements are promising for testing many additives or comparing several dietary treatments as concern their effects on rumen fermentability (in terms of GP) and methane production (MP) (Yáñez-Ruiz et al., 2016). Pellikaan et al. (2011) and Ramin and Huhtanen (2012) suggested that the kinetics of methane production in vitro differ from that of GP and tested a manual batch fermentation system suitable for several samplings of gases.

In the currently available apparatus for the simultaneous measurement of GP and MP, the gas accumulation in the fermenters can generate an increase in pressure causing a change in gas (CO_2) dissolution. This represents a potential disrupter in the fermentation process with a consequent complication in gas production assessment (Alvarez Hess et al., 2018; Cattani et al., 2014). Therefore, the methane concentration is measured at the end of fermentation in apparatus capable of accumulating gas in growing volumes (Menke & Steingass, 1988) or from the gas that is regularly released from fermentation bottles in vented systems. Muetzel et al. (2014) first developed an automated batch fermentation system for methane assessment with bottles fitted with mechanized systems of valves, pressure detectors, and devices to provide gas venting and sampling.

Applying an infrared (IR) sensor to monitor MP in the outflowing gas throughout the entire fermentation process is a further advancement because gas sampling is not required and the continuous MP measure allows accurate kinetic studies. In the present work, we test a new apparatus based on IR detection, made up of independent units (fermentation bottles with gas counters and detector) installed in parallel to allow multiple simultaneous tests.

The aim of this work is to describe the batch fermentation system and to study the kinetics of MP in comparison to that of total GP. We hypothesize that the continuous measurement of methane in batch systems provides more accurate data than that obtained from a few sampling points during fermentation.

2 | MATERIALS AND METHODS

2.1 | Apparatus description

The apparatus (Figure 1) is composed of fermentation glass bottles (total available capacity 750 ml) closed with an airtight cap equipped with a mixing system (rotation speed 18 rpm). Each bottle is connected with a flexible plastic tube (inner diameter 4 mm) to the gas counter (Ritter Apparatebau GmbH & Co. KG). This is composed of a bar immersed in oil that changes position at each entrance of 3 ml of gas and every bar movement was registered by a computer. After the volume measurement, the gas flows through a plastic tube into the IR gas analyser (RI. sens mono IR1; Ritter Apparatebau GmbH & Co. KG) for measuring the methane concentration. The analyser is calibrated to detect methane concentrations from 0% to 30% of the total gas (accuracy ±2%) at temperatures from 5°C to 45°C. The IR sensor works at pressures ranging from 800 to 1200 mbar and it was previously tested with standard gas to guarantee measurement accuracy. When the MP was estimated, the volume of the connecting tubes (10 ml) combined with the volume in the upper part of the fermenter (250 ml) was considered as headspace volume (total headspace volume 260 ml).

In the present experiment, eight bottles were filled with filtered rumen fluid and mixed with the Menke and Steingass (1988) buffer (ratio 1:2, 500 ml in total). Substrates (3300 mg of dry matter [DM]) were weighed and introduced into each bottle as ground and dry materials and then bottles were closed and immersed in a water bath at 39°C for 48 h.

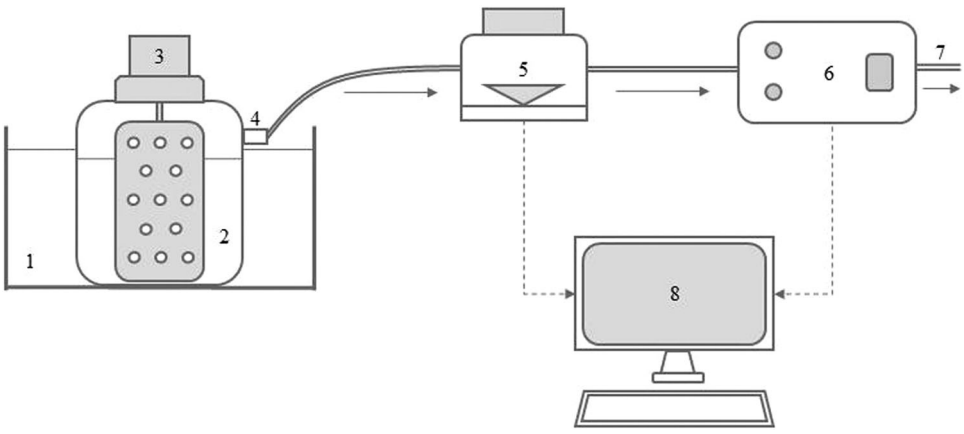

FIGURE 1 Layout of the fermentation system unit: 1, water bath; 2, fermentation bottle containing rumen fluid and buffer solution; 3, stirring device; 4, gas outlet hole; 5, gas flow counter; 6, methane infrared analyser; 7, gas discharge; 8, computer connected for the data collection. The arrows indicate the gas flow, while the dashed arrows indicate the line of data acquisition.

2.2 | Substrates and fermentation runs

Four different feeds for ruminants, namely, barley meal (BM), alfalfa hay (AH), corn silage, (CS), and soya bean hulls (SH), were tested in four consecutive fermentation runs lasting 48 h. CS was dried (48 h at 60°C) and then all the feeds were milled and analysed in duplicate for analytical DM, crude protein (CP), neutral detergent fibre (Mertens, 2002), and ash content according to the instructions of the Association of Official Analytical Chemists (1995).

The rumen fluid for all of the fermentation runs was collected in the same slaughterhouse in controlled conditions: mixed fluid was collected within 20 min of slaughter from four culled dairy cows fed with total mixed rations based on CS; no cow was slaughtered in an emergency; each cow was in good health, and all of the cows were transported from farms located within 50 km of the slaughterhouse. The fluid was delivered, within half an hour of it being collected, to the laboratory in airtight glass bottles refluxed with CO_2 and maintained at 39°C.

2.3 | Fermentation fluid sampling and analysis

At the end of the incubation, pH was directly measured (GLP 22; Crison Instruments), while samples for NH_3 and volatile fatty acid (VFA) analyses were taken and stored at −20°C until the analyses were carried out.

Ammonia–nitrogen samples were thawed at room temperature and analysed using an ammonia electrode (Ammonia Gas Sensing Combination Electrode; Hach Company; 2001). Samples for VFA analysis, to each of which a volume of 5 ml of 0.01 mol/L H_2SO_4 was previously added, were thawed at room temperature, centrifuged at 20,000*g* for 20 min at 4°C, and filtered using a polypore filter (0.45 mm; Agilent Technologies). The filtrate was injected into a high-performance liquid chromatography instrument (PerkinElmer) with its analysis wavelength set to 220 nm. The VFA concentration was measured as described by Martillotti and Puppo (1985).

2.4 | Calculations and fitting

The cumulative MP was calculated using the following equation obtained by adapting to our system, which was suggested by Mengistu et al. (2017):

$$MP \quad (\text{ml}) = \sum_{i=1}^{i=n+1} \left((C_{i+1} - C_i) \times \frac{260}{100} \right) + \left(\frac{(C_{i+1} + C_i)}{2} \times \frac{\Delta V}{100} \right),$$

In this equation, C_i and $C_i + 1$ are the methane concentrations measured at time $i + 1$ and i, respectively, ΔV is the difference between the volume of gas (ml) produced at $i + 1$ and that at i, and n is the total number of methane detections. The cumulative MP was calculated considering the variation of methane concentration and the increase in volume between hour intervals. Cumulative volumes of GP and MP and the percentage of methane in the total GP were recorded continuously for 48 h and average values at 1 h intervals were fitted with an exponential model with lag phase, specifically using the equation:

$$y = A \times (1 - \exp^{(-k \times (t-L))}),$$

where y is the dependent variable at time t (h), A (ml) is the asymptotic GP or MP values, k (h^{-1}) is a rate constant of GP or MP production, and L is the lag time (h).

2.5 | Statistical analysis

The fermentation runs ($n = 4$) were completed in sequenced periods (weeks) and data from two fermentation bottles within a run were averaged and the mean was used as a statistical unit (replicates among runs).

The pH, the amounts of NH_3 and VFAs, the relative percentage of single VFA, the kinetic parameters for GP, MP, and methane percentage in GP, the total amount of methane calculated from concentration measured at the end of fermentation (48 h) and that calculated at multiple sampling points during fermentation (at 1, 2, 3, 4, 6, 8, 24, and 48 h) were statistically analysed with a factorial randomized complete block (fermentation run) design using the equation:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where Y_{ij} is the experimental data, μ is the overall mean, α_i is the random effect (block) of the fermentation run (i= 1,4); β_j is the fixed effect of the substrate (j = 1,4), and ε_{ij} is the residual error. Statistical analyses were performed with SAS software (version 9.4; SAS Institute).

3 | RESULTS AND DISCUSSION

In the present experiment, the rumen fluid used as inoculum in the different fermentative runs was collected at the slaughterhouse during four sessions. In each sampling, fluids from several healthy dairy cows fed similar diets were mixed to limit rumen inoculum variability. As a result, we obtained some fermentative metrics, such as GP or rate of gas production, a variability due to different collection sessions smaller (around one-fourth) than that attributable to various feeds.

The main aim of this work was to study the kinetics of MP and for this purpose; the total gas and methane were fitted without subtracting values from blank incubation. In fact, according to Cone (1998), blank values do not produce gas at the same rate as samples during incubations because microbial turnover begins earlier, and this phenomenon may cause an error in fermentation dynamics calculation.

The system tested differs from previously proposed in other scientific papers since the application of an IR sensor allows direct and continuous detection of methane concentrations in the gas flowing without accumulation in the fermentation bottle. This

prevents modification in CO_2 dissolution in the liquid phase and any disruption of the fermentation caused by increased pressure during fermentation, as demonstrated in the literature (Tagliapietra et al., 2010). This system appears convenient because it does not require complex equipment such as valves, pressure detectors, and related recording systems to manage outflow gas. The proximal composition of feeds used the fermentative parameters after 48 h of incubation and the kinetics parameters obtained for the different feeds are reported in Table 1.

For AH incubation, rumen fluid shows a higher ammonia concentration and pH after 48 h of incubation ($p < 0.01$) compared to other feeds, owing to the high CP content.

TABLE 1 Chemical composition of substrates, fermentation data (NH_3, pH, VFAs) of fluids at the end of fermentation (48h) and kinetic parameters of total gas and methane production .

	Barley meal	Alfalfa hay	Corn silage	Soybean hulls	RMSE
Chemical composition					
Crude protein (%DM)	12.4	18.3	7.6	13.3	
NDF (%DM)	17.9	48.5	36.5	67.3	
Ash (%DM)	2.4	9.4	3.9	4.7	
Fermentation parameters					
pH	6.56^{B}	6.77^{A}	6.56^{B}	6.52^{C}	0.017
NH_3 (mg/dl)	52.0^{AB}	53.7^{A}	49.4^{C}	50.3^{AB}	1.124
Total VFA (mmol/L)	59.62^{A}	51.06^{C}	53.71^{B}	59.72^{A}	1.537
% total VFA					
Acetate	65.18^{B}	68.95^{A}	65.50^{B}	68.67^{A}	1.274
Propionate	12.73^{b}	13.80^{ab}	14.63^{a}	15.29^{a}	0.880
Isobutyrate	1.30	1.48	1.31	1.31	0.169
Butyrate	16.31^{A}	11.35^{C}	14.09^{B}	10.86^{C}	1.299
Isovalerate	3.38^{ab}	3.74^{a}	3.57^{ab}	3.09^{b}	0.241
Valerate	1.12^{A}	0.97^{AB}	0.92^{BC}	0.76^{C}	0.101
Acetate:propionate	5.22	5.06	4.52	4.54	0.369
Gas and methane production					
Cumulative total gas					
A (ml)	1520^{B}	1000^{D}	1437^{C}	1836^{A}	48.06
k (h^{-1})	0.070^{B}	0.098^{A}	0.071^{B}	0.042^{C}	0.008
Lag (h)	0.000^{B}	0.000^{B}	0.003^{B}	0.278^{A}	0.096
R^2	>0.999	>0.999	>0.999	0.992	
Cumulative methane					
A (ml)	314^{B}	233^{C}	295^{B}	370^{A}	16.52
k (h^{-1})	0.085^{A}	0.074^{B}	0.066^{B}	0.042^{C}	0.006
Lag (h)	0.212^{B}	0.094^{B}	0.352^{B}	1.120^{A}	0.248
R^2	0.996	>0.999	0.998	0.994	
Methane (%)					
A	19.24^{B}	24.94^{A}	21.19^{B}	20.90^{B}	1.249
k	0.743^{A}	0.200^{C}	0.293^{B}	0.230^{BC}	0.043
Lag	0.022	0.067	0.260	0.268	0.119
R^2	0.904	0.994	0.947	0.956	

Note: R^2: (1 − (residual sum of squares)/(total sum of squares)); $^{a-b-c,\ A-B-C}$ within rows, means without a common superscript differ ($p < 0.05$, $p < 0.01$).
Abbreviations: DM, dry matter; NDF, neutral detergent fibre; RMSE, root mean square error; VFA, volatile fatty acid.

The total VFA concentration in the fluid resulting from the use of BM and SH (59.6–59.7 mmol/L) was approximately 20% higher than in the fluid resulting from the use of AH and CS (51.1–53.7 mmol/L). The VFA composition differed significantly between substrates, with high variations in the relative amount of butyrate (from 10.9 to 16.3% of the total VFA; $p < 0.01$), lower variations in propionate (from 12.7% to 15.3% of the total VFA; $p < 0.05$), and small but significant variations in acetate (from 65.2% to 68.9% of the total VFA; $p < 0.01$).

A representation of the kinetics is shown in Figure 2. Both the GP and MP kinetics were very well fitted with the exponential model described above ($R^2 > 0.992$). A discrete *L* for MP (from 0.09 to 1.12 h) was observed, but no lag for the total GP was found (except for SH).

The kinetic parameters describing the changes in cumulative total GP and MP and the percentage of methane in the total gas produced are reported in Table 1. GP and MP plateaued at the highest levels when using SH as feed (1836 and 370 ml, respectively; $p < 0.01$), at the lowest for AH (1000 and 233 ml, respectively), and the remaining substrates showed intermediate values.

The asymptotic methane concentrations we observed ranged between 19.9% to 21.9% for the four feeds and were close to those obtained by Mengistu et al. (2017), who tested compound feed, grass, and CS at the same duration of in vitro fermentation. The concentrations we observed were slightly higher than the 17.0%–21.1% values found by Pellikaan et al. (2011), who used various common types of feed for 72 h of in vitro fermentation. However, Maccarana et al. (2016) indicated that methane

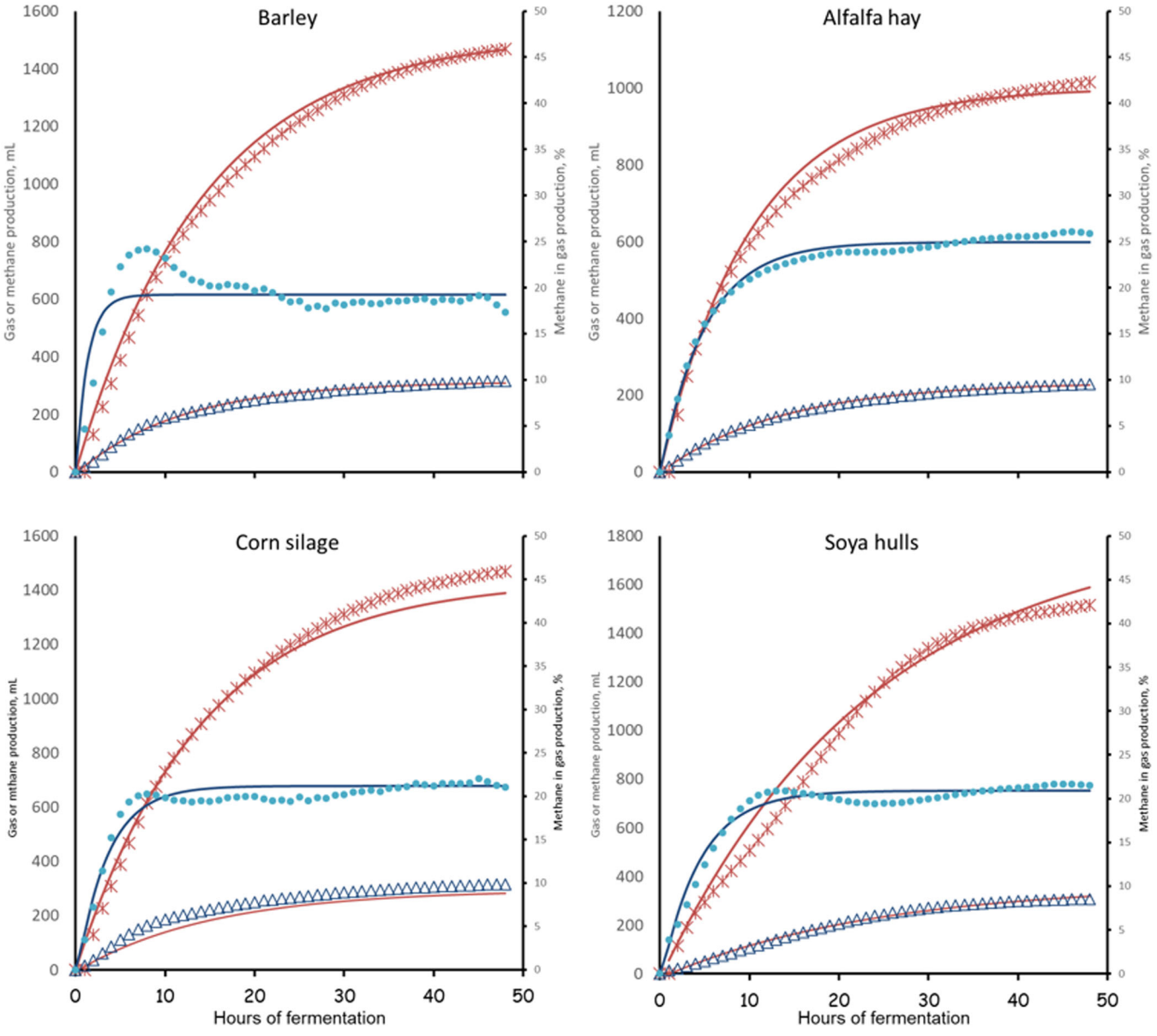

FIGURE 2 Kinetics (lines) and data points (symbols) of the cumulative volumes of gas and methane (left *y*-axis, ✳ and Δ, respectively) and the percentage of methane in the total gas (right *y*-axis, ●). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 MP was calculated using total GP and the percent of methane measured at the conclusion of fermentation (48 h) or at multiple sampling points during fermentation (eight sampling points)

	Barley	Alfalfa hay	Corn silage	Soybean hulls	RMSE
Methane production					
Endpoint measure[a] (ml)	333[B]	312[B]	350[AB]	385[A]	22.4
Multiple measures[b] (ml)	316[AB]	245[C]	298[B]	338[A]	16.8
Difference (%)	5.3[C]	27.8[A]	17.6[B]	14.1[B]	3.22

Note: A–B–C within rows, means without a common superscript differ ($p < 0.01$).

Abbreviation: RMSE, root mean square error.

[a]Total gas and methane measured at the end of fermentation (48 h).

[b]Total gas and methane measured at 1, 2, 3, 4, 6, 8, 24, and 48 h from the beginning of the fermentation.

concentration measurements have been highly influenced by the methodological protocol adopted. These authors carried out a meta-analysis of 30 papers and showed the relative amount of methane in the total gas produced to be on average 17.0% but with very high variability (Standard deviation: ±7.5% point) between the different experiments.

Navarro-Villa et al. (2011) measured the MP in straw, grass, and grain and concluded that in vitro batch systems are not appropriate to rank feeds based on expected MP due to the biochemistry of rumen fermentation. In fact, the greater availability of H_2 for methanobacteria in fibrous feeds affects methane production (Ungerfeld, 2020). One possible explanation is that the in vitro systems are highly buffered avoiding the pH drop, which normally occurs in in vivo conditions with starchy, feeds, and is associated with reduced cellulolytic and methanogen activities.

The methane concentration in gas flowing changed during the fermentation: the percentage of methane increased rapidly (from 0.35 to 0.95 h^{-1}) initially and began to plateau at approximately 8–12 h. Furthermore, this process seemed to be substrate-dependent because plateaus were reached at different rates for the four substrates ($p < 0.01$).

The low relative methane amount detected at the beginning of the fermentation was consistent with the findings of Menci et al. (2021), who discovered a methane concentration of 9%–10% after 3.5 h of fermentation with an increase up to 28%–30% after 24 h. Colombini et al. (2021) measured only a slight increase in methane concentration (from 20%–21% to 23%–24%) during the second half of fermentation (from 24 to 48 h), whereas Muetzel et al. (2014) took 20–25 measurements of gas volume and composition, with more than 60% of the measurements taken during the first 12 h of fermentation. These authors found a delay in the MP in the early stages of the fermentation, with half of the total generated methane produced approximately 3–4 h after half of the total generated gas was produced.

In the present experiment, a delay in the MP compared with GP was clearly observed and this phenomenon may be caused by several factors. The methanogenesis follows the hydrolytic attack of polysaccharides (in particular cellulose, hemicellulose, and starch) by micro-organisms and the metabolism of the resulting monosaccharides into VFAs and CO_2. While in in vivo and in continuous fermentation systems there is a permanent supply of H_2 for the methanobacteria, in an in vitro batch system, the H_2 becomes progressively more available after the start of the polysaccharide fermentation.

Based on our experimental data set, a comparison between the total methane calculated from a single measurement after 48 h of fermentation (endpoint measure) and that obtained using multiple measurements (eight sampling points) was performed to assess the practical significance of delay in methane production. For the endpoint measure, total methane was calculated using the concentration of methane reached at the end of fermentation and the total gas produced, whereas for multiple measurements, the equation previously described was adopted. According to the results reported in Table 2, MP calculated from a single endpoint measurement overestimates total methane produced by 5%–28% when compared to MP calculated from multiple measurements. The overestimation error varied greatly between substrates, with BM having the lowest error (5%), CS and SM having intermediate error values (14%–18%), and AH having the highest error (28%). Unfortunately, such variation in error makes it impossible to apply a constant correction.

In conclusion, the rumen fermentation system evaluated generates methane data comparable to those reported in the literature and allows simple continuous measurement of methane release throughout fermentation. Since the kinetics of methane and total gas production are different, the equipment under study is an accurate metric for methane assessment.

4 | ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

ACKNOWLEDGEMENTS

Open Access Funding provided by Universita degli Studi di Udine within the CRUI-CARE Agreement.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Matteo Braidot http://orcid.org/0000-0003-1433-7432
Chiara Sarnataro http://orcid.org/0000-0002-7924-2357
Alberto Romanzin https://orcid.org/0000-0001-9750-0607
Mauro Spanghero https://orcid.org/0000-0001-9782-8194

REFERENCES

Alvarez Hess, P. S., Eckard, R. J., Jacobs, J. L., Hannah, M. C., & Moate, P. J. (2018). Comparison of five methods for the estimation of methane production from vented in vitro systems. *Journal of the Science of Food and Agriculture*, *99*(1), 109–116. https://doi.org/10.1002/jsfa.9149

Association of Official Analytical Chemists. (1995). *Official methods of analysis of the Association of Official Analytical Chemists*.

Cattani, M., Tagliapietra, F., Maccarana, L., Hansen, H. H., Bailoni, L., & Schiavon, S. (2014). Technical note: In vitro total gas and methane production measurements from closed or vented rumen batch culture systems. *Journal of Dairy Science*, *97*(3), 1736–1741. https://doi.org/10.3168/jds.2013-7462

Colombini, S., Rota Graziosi, A., Parma, P., Iriti, M., Vitalini, S., Sarnataro, C., & Spanghero, M. (2021). Evaluation of dietary addition of 2 essential oils from *Achillea moschata*, or their components (bornyl acetate, camphor, and eucalyptol) on in vitro ruminal fermentation and microbial community composition. *Animal Nutrition*, *7*(1), 224–231. https://doi.org/10.1016/j.aninu.2020.11.001

Commission of the European Communit. (2020). *Communication from the commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions: On an EU strategy to reduce methane emissions*.

Cone, J. W. (1998). The development, use and application of the gas production technique at the DLO Institute for Animal Science and Health (ID-DLO), Lelystad, the Netherlands. *BSAP Occasional Publication*, *22*, 65–78. https://doi.org/10.1017/S0263967X00032286

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock—A global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations (FAO).

Maccarana, L., Cattani, M., Tagliapietra, F., Schiavon, S., Bailoni, L., & Mantovani, R. (2016). Methodological factors affecting gas and methane production during in vitro rumen fermentation evaluated by meta-analysis approach. *Journal of Animal Science and Biotechnology*, *7*(1), 35. https://doi.org/10.1186/s40104-016-0094-8

Martillotti, F., & Puppo, P. (1985). Liquid chromatographic determination of organic acids in silages and rumen fluids. *Annuali Istituto Superiore di Zootecnia*, *18*, 1–10.

Menci, R., Coppa, M., Torrent, A., Natalello, A., Valenti, B., Luciano, G., Priolo, A., & Niderkorn, V. (2021). Effects of two tannin extracts at different doses in interaction with a green or dry forage substrate on in vitro rumen fermentation and biohydrogenation. *Animal Feed Science and Technology*, *278*, 114977. https://doi.org/10.1016/j.anifeedsci.2021.114977

Mengistu, G., Hendriks, W. H., & Pellikaan, W. F. (2017). In vitro methane and gas production with inocula from cows and goats fed an identical diet. *Journal of the Science of Food and Agriculture*, *98*(4), 1332–1338. https://doi.org/10.1002/jsfa.8597

Menke, K. H., & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development*, *28*, 7–55.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC International*, *85*, 1217–1240.

Muetzel, S., Hunt, C., & Tavendale, M. H. (2014). A fully automated incubation system for the measurement of gas production and gas composition. *Animal Feed Science and Technology*, *196*, 1–11. https://doi.org/10.1016/j.anifeedsci.2014.05.016

Navarro-Villa, A., O'Brien, M., López, S., Boland, T. M., & O'Kiely, P. (2011). Modifications of a gas production technique for assessing in vitro rumen methane production from feedstuffs. *Animal Feed Science and Technology*, *166–167*, 163–174. https://doi.org/10.1016/j.anifeedsci.2011.04.064

Pellikaan, W. F., Hendriks, W. H., Uwimana, G., Bongers, L. J. G. M., Becker, P. M., & Cone, J. W. (2011). A novel method to determine simultaneously methane production during in vitro gas production using fully automated equipment. *Animal Feed Science and Technology*, *168*(3-4), 196–205. https://doi.org/10.1016/j.anifeedsci.2011.04.096

Ramin, M., & Huhtanen, P. (2012). Development of an in vitro method for determination of methane production kinetics using a fully automated in vitro gas system—A modelling approach. *Animal Feed Science and Technology*, *174*(3-4), 190–200. https://doi.org/10.1016/j.anifeedsci.2012.03.008

Tagliapietra, F., Cattani, M., Bailoni, L., & Schiavon, S. (2010). In vitro rumen fermentation: Effect of headspace pressure on the gas production kinetics of corn meal and meadow hay. *Animal Feed Science and Technology*, *158*(3-4), 197–201. https://doi.org/10.1016/j.anifeedsci.2010.04.003

Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. *Frontiers in Microbiology*, *11*, 589. https://doi.org/10.3389/fmicb.2020.00589

Yáñez-Ruiz, D. R., Bannink, A., Dijkstra, J., Kebreab, E., Morgavi, D. P., O'Kiely, P., Reynolds, C. K., Schwarm, A., Shingfield, K. J., Yu, Z., & Hristov, A. N. (2016). Design, implementation and interpretation of in vitro batch culture experiments to assess enteric methane mitigation in ruminants—A review. *Animal Feed Science and Technology*, *216*, 1–18. https://doi.org/10.1016/j.anifeedsci.2016.03.016

How to cite this article: Braidot, M., Sarnataro, C., Romanzin, A., & Spanghero, M. (2022). A new equipment for continuous measurement of methane production in a batch in vitro rumen system. *Journal of Animal Physiology and Animal Nutrition*, 1–7. https://doi.org/10.1111/jpn.13780