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In vitro cumulative gas production techniques: History, methodological considerations and challenges

C. Rymer [a,*], J.A. Huntington [b], B.A. Williams [c], D.I. Givens [a]

[a] *Animal Science Research Group, School of Agriculture, Policy and Development, University of Reading, P. O. Box 237, Reading RG6 6AR, UK*
[b] *Harper Adams University College, Newport TF10 8NB, UK*
[c] *Animal Nutrition Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands*

Abstract

Methodology used to measure in vitro gas production is reviewed to determine impacts of sources of variation on resultant gas production profiles (GPP). Current methods include measurement of gas production at constant pressure (e.g., use of gas tight syringes), a system that is inexpensive, but may be less sensitive than others thereby affecting its suitability in some situations. Automated systems that measure gas production at constant volume allow pressure to accumulate in the bottle, which is recorded at different times to produce a GPP, and may result in sufficiently high pressure that solubility of evolved gases in the medium is affected, thereby resulting in a recorded volume of gas that is lower than that predicted from stoichiometric calculations. Several other methods measure gas production at constant pressure and volume with either pressure transducers or sensors, and these may be manual, semi-automated or fully automated in operation. In these systems, gas is released as pressure increases, and vented gas is recorded. Agitating the medium does not consistently produce more gas with automated systems, and little or no effect of agitation was observed with manual systems. The apparatus affects GPP, but mathematical manipulation may enable effects of apparatus to be removed. The amount of substrate affects the volume of gas produced, but not rate of gas production, provided there is sufficient buffering capacity in the medium. Systems that use a very small amount of substrate are prone to experimental error in sample weighing. Effect of sample preparation on GPP has been found to be important, but further research is required to determine the

Abbreviations: DM, dry matter; GPP, gas production profile; NDF, neutral detergent fibre; OM, organic matter; SCFA, short chain fatty acids

* Corresponding author. Tel.: +44 118 3786436; fax: +44 118 3786595.
E-mail address: c.rymer@reading.ac.uk (C. Rymer).

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optimum preparation that mimics animal chewing. Inoculum is the single largest source of variation in measuring GPP, as rumen fluid is variable and sampling schedules, diets fed to donor animals and ratios of rumen fluid/medium must be selected such that microbial activity is sufficiently high that it does not affect rate and extent of fermentation. Species of donor animal may also cause differences in GPP. End point measures can be mathematically manipulated to account for species differences, but rates of fermentation are not related. Other sources of inocula that have been used include caecal fluid (primarily for investigating hindgut fermentation in monogastrics), effluent from simulated rumen fermentation (e.g., 'Rusitec', which was as variable as rumen fluid), faeces, and frozen or freeze-dried rumen fluid (which were both less active than fresh rumen fluid). Use of mixtures of cell-free enzymes, or pure cultures of bacteria, may be a way of increasing GPP reproducibility, while reducing reliance on surgically modified animals. However, more research is required to develop these inocula. A number of media have been developed which buffer the incubation and provide relevant micro-nutrients to the microorganisms. To date, little research has been completed on relationships between the composition of the medium and measured GPP. However, comparing GPP from media either rich in N or N-free, allows assessment of contributions of N containing compounds in the sample.

Keywords: Gas production; Feed characterisation; Rumen in vitro methodology

1. Introduction

In vitro cumulative gas production techniques were developed to predict fermentation of ruminant feedstuffs. A feedstuff is incubated with buffered rumen fluid and gas produced is measured as an indirect indicator of fermentation kinetics. When a feedstuff is incubated with buffered rumen fluid, it is first degraded and the degraded fraction may either be fermented to produce gas and fermentation acids, or incorporated into microbial biomass. When combined with measures of degradation, gas production techniques provide a measure of the proportion of feed that is fermented as opposed to that which is partitioned to microbial growth.

The principle of determining potential rumen degradability/fermentability of a feed by measuring gas produced from a batch culture was first developed by McBee (1953) and Hungate (1966). Trei et al. (1970) adapted the earlier techniques by attaching a water displacement manometer to each vessel to measure the gas produced. Similarly, Jouany and Thivend (1986) and Beuvink and Spoelstra (1992) used inverted measuring cylinders to determine the volume of water displaced. Beuvink et al. (1992) then automated this water displacement technique.

Direct displacement of a plunger by fermenting a feedstuff within a glass syringe was developed by Czerkawski and Breckenridge (1975) and was the basis of the 'Hohenheim Gas Test' later developed by Menke et al. (1979). Blümmel and Ørskov (1993) modified the technique by incubating syringes in a waterbath rather than a rotating incubator. The syringe technique was originally developed to determine end-point fermentability of feedstuffs, at 24 h, and has been incorporated into the German national feedstuff evaluation system. However, by recording plunger displacement at more frequent intervals, the kinetics of the fermentation profile can also be determined.