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# Inter-laboratory variation of in vitro cumulative gas production profiles of feeds using manual and automated methods

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**Abstract**

A study was conducted to estimate variation among laboratories and between manual and automated techniques of measuring pressure on the resulting gas production profiles (GPP). Eight feeds (molassed sugarbeet feed, grass silage, maize silage, soyabean hulls, maize gluten feed, whole crop wheat silage, wheat, glucose) were milled to pass a 1 mm screen and sent to three laboratories (ADAS Nutritional Sciences Research Unit, UK; Institute of Grassland and Environmental Research (IGER), UK; Wageningen University, The Netherlands). Each laboratory measured GPP over 144 h using standardised procedures with manual pressure transducers (MPT) and automated pressure systems (APS). The APS at ADAS used a pressure transducer and bottles in a shaking water bath, while the APS at Wageningen and IGER used a pressure sensor and bottles held in a stationary rack. Apparent dry matter degradability (ADDM) was estimated at the end of the incubation. GPP were fitted to a modified Michaelis–Menten model assuming a single phase of gas production, and GPP were described in

*Abbreviations: A*, total volume of gas produced (ml/g DM incubated); ADDM, apparent dry matter degradability in vitro; APS, automated pressure system; CP, crude protein; DM, dry matter; EE, ether extract; GLU, glucose; GPP, gas production profile; GS, grass silage; *B*, time to half *A* (h); MGF, maize gluten feed; MPT, manual pressure transducer; MS, maize silage; MSBF, molassed sugarbeet feed; NDF, neutral detergent fibre; OM, organic matter; $R_{M\,gas}$, maximum gas production rate (ml/g DM incubated h); SBH, soyabean hulls; $t_{RM\,gas}$, time to maximum gas production rate (h); WCW, ensiled whole crop wheat; WHT, wheat

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terms of the asymptotic volume of gas produced ($A$), the time to half $A$ ($B$), the time of maximum gas production rate ($t_{RM\,gas}$) and maximum gas production rate ($R_{M\,gas}$). There were effects ($P<0.001$) of substrate on all parameters. However, MPT produced more ($P<0.001$) gas, but with longer ($P<0.001$) B and $t_{RM\,gas}$ ($P<0.05$) and lower ($P<0.001$) $R_{M\,gas}$ compared to APS. There was no difference between apparatus in ADDM estimates. Interactions occurred between substrate and apparatus, substrate and laboratory, and laboratory and apparatus. However, when mean values for MPT were regressed from the individual laboratories, relationships were good (i.e., adjusted $R^2 = 0.827$ or higher). Good relationships were also observed with APS, although they were weaker than for MPT (i.e., adjusted $R^2 = 0.723$ or higher). The relationships between mean MPT and mean APS data were also good (i.e., adjusted $R^2 = 0.844$ or higher). Data suggest that, although laboratory and method of measuring pressure are sources of variation in GPP estimation, it should be possible using appropriate mathematical models to standardise data among laboratories so that data from one laboratory could be extrapolated to others. This would allow development of a database of GPP data from many diverse feeds.


*Keywords:* Gas production; Ring test; Feed characterisation; Manual pressure transducer; Automated pressure system

## 1. Introduction

In vitro cumulative gas production techniques were originally developed as means of obtaining information on the dynamics of rumen fermentation of feeds. Kinetic estimates from gas production data have been transformed to inputs for mathematical models describing ruminant physiology (Pitt et al., 1999). Brown et al. (2002) and Rymer and Givens (2002) observed that they were also well related to in vivo measures of patterns of rumen fermentation, such as pH and the relative proportions of individual short chain fatty acids.

However, from a practical perspective, there are a number of sources of variation in estimation of a gas production profile (GPP). These include the apparatus used (Rymer and Givens, 1997; Lowman et al., 1998; López et al., 1998), the species of inoculum donor (Calabrò et al., 2004; Cone et al., 2002), animal diet (Cone et al., 1996), rumen inoculum sampling site (Muetzel et al., 2001), and preparation of both the rumen fluid (Pell and Schofield, 1993; Rymer et al., 1999) and substrate (Menke and Steingass, 1988; Deaville, 1995; Williams et al., 1995; Lowman et al., 2002; DePeters et al., 2003; Akyol et al., 2004). Comparing results among laboratories requires an indication of the extent of variation among them, and effects of the method of pressure measurement on the GPP produced. Under standardised conditions, acceptable repeatability has been observed among laboratories using the same apparatus (Menke and Steingass, 1988; van Gelder et al., 2005). However, there is wide variation in the apparatus used to generate GPP, ranging from syringes (Menke and Steingass, 1988), the manual pressure transducer (MPT) developed by Theodorou et al. (1994), and the automated systems (APS) described by Pell and Schofield (1993), Cone et al. (1996) and Davies et al. (2000).

The objectives of this experiment were to compare GPP produced by a MPT with the automated systems of Davies et al. (2000) and Cone et al. (1996), and to compare variation among laboratories in GPP obtained using these apparatus when standardised conditions were applied.