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Short communication

Comparison of intestinal contents from different regions of the equine gastrointestinal tract as inocula for use in an *in vitro* gas production technique

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ABSTRACT

The aim of the experiment reported here was to compare equine faeces with inocula obtained from equine caecum, ventral colon and dorsal colon for use in the *in vitro* gas production technique. Freeze-dried grass (FDG), high-temperature dried grass (HDG) and unmolassed sugar beet pulp (BP) were incubated with either caecal fluid (C), dosal colonic fluid (D), ventral colonic fluid (V) or faeces (F) as the source of inoculum. Substrate/inocula combinations were fermented using an *in vitro* gas production (GP) technique. Mathematical analysis of cumulative gas production curves revealed an interaction ($P<0.001$) between inocula and substrate for all of the *in vitro* parameters measured. However, similar extents of gas production were seen for inocula obtained from caecal fluid and faeces across all substrates, with lower values obtained from bottles incubated with colonic fluid. Thus, results indicate that faeces are a suitable alternative to caecal fluid; however, it would appear that they are less effective in modelling fermentation in the colon. Consequently, further work is required to determine the use of faeces as an alternative to caecal and colonic fluid and to further investigate inocula/substrate interactions.

1. Introduction

In vitro techniques offer the potential to ascertain the nutritive value of feedstuffs as an alternative to *in vivo* experiments. The *in vitro* gas production technique (IVGPT) allows for both the rate and extent of degradation of feedstuff to be assessed, that latter being of significance to the horse due to the more rapid total tract transit time in horses compared to ruminants (McLean et al., 1995; Drogoul et al., 2001; Moore-Colyer et al., 2003). The IVGPT relies on an anaerobic medium, feed substrate and a representative sample of the micro-organisms present in the areas of the gastrointestinal (GI) tract in which fermentation occurs (inoculum). The microbial inoculum has been reported as being one of the largest sources of variation in the IVGPT (Hervas et al., 2005). Variations in microbial inocula activity have been attributed to sampling time/day, donor animal and its diet, storage, inoculum preparation and concentration, and inocula source (Mould et al., 2005). The IVGPT

Abbreviations: A, asymptotic gas production; B, half time of asymptotic gas production; BP, unmolassed sugar beet pulp; FDG, freeze-dried grass; GI, gastrointestinal; GP, gas production; HDG, high-temperature dried grass; IVGPT, in vitro gas production technique; MFR, maximal fractional rate of substrate degradation; t_I, inflection point.

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was originally developed to evaluate the fermentation of feedstuffs for ruminants and relied on rumen fluid as the source of microbial inoculum (Theodorou et al., 1994). Although the digestive tracts of ruminants and equids have followed different evolutionary pathways, the objective of both is to process forages efficiently (Janis, 1976). Consequently, the IVGPT has been used to evaluate feedstuffs for post-gastric fermenters such horses (Murray et al., 2005a, 2006, 2009).

Obtaining caecal or rumen fluid requires either surgical modification or euthanasia. Consequently, many studies using the IVGPT in post-gastric fermenters have involved the use of faeces as the source of inoculum. The majority of micro-organisms in the mammalian digestive tract are not attached to the GI tract itself, but instead adhere to food particles travelling through the tract or remain suspended in the digesta. Hence, gut micro-organisms that are closely associated with plant debris in the GI tract are also excreted in the faeces. Faecal material remains largely anaerobic after voiding and thus the microbes can remain viable for several hours after excretion from the digestive tract (Holter, 1991). Consequently, many studies have involved the use of faeces as an alternative to rumen or caecal fluid (El Shaer et al., 1987; Akhter, 1994; Mauricio et al., 1998; Akhter et al., 1999; Mould et al., 2005).

However, whilst there has been considerable research into the use of faeces from ruminants as an alternative to rumen fluid (Mould et al., 2005), there has been little work done to compare equine faeces with inocula obtained from different fermentative regions of the equine GI tract. Consequently, the aim of the experiment reported here was to compare equine faeces with inocula obtained from equine caecum, ventral colon and dorsal colon for use in the *in vitro* gas production technique of Theodorou et al. (1994).

2. Materials and methods

Three 160 ml identical series of serum bottles were used to assess the fermentation characteristics of 600 mg DM (±0.5%) of ground (to pass through a 1 mm dry mesh screen) freeze-dried grass (FDG), high-temperature dried grass (HDG) and unmolassed sugar beet pulp (BPBP) incubated with either caecal fluid (C), dorsal colonic fluid (D), ventral colonic fluid (V) or faeces (F) as the source of inoculum. *In vitro* fermentations were conducted according to the method described by Theodorou et al. (1994) with the following modifications. The inocula were prepared from the GI tract contents obtained from a horse immediately following euthanasia. The donor animal was a patient at the Royal (Dick) School of Veterinary Studies and had been euthanised for non-GI tract-related illness. The animal was on a diet of *ad lib* hay with a small concentrate ration (approx. 1 kg/d). Digesta was obtained from the caecum, ventral and dorsal colons along with a sample of faeces from the rectum. Inocula were placed in separate airtight containers for immediate transportation immediately to the laboratory. Caecal and colonic samples were strained through a triple layer of muslin. The faecal inoculum was prepared by combining faeces with an equal weight of culture medium, then homogenised in a stomacher (Laboratory blender stomacher 400, Seward, London, UK) for 90 s. The resultant suspension was strained through a triple layer of muslin and collected in a CO_2 filled flask. The resultant inocula were placed in pre-warmed flasks and incubated at 38 °C under anaerobic conditions prior to inoculation. *In vitro* fermentation bottles were prepared with 60 ml culture media and 30 ml inoculum per bottle.

The experiment was a factorial design consisting of three different substrates, four different sources of inocula and three replicate bottles. Thus, a total of 48 bottles were included for GP; 36 containing substrate (3 per substrate/inocula combination) and 12 inocula blanks (no substrate; 3 per inocula source). Head-space gas pressure readings were taken at 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 36, 48, 56, and 72 h post-inoculation, with the accumulated gas volume measured and then released to zero after each reading (Fig. 1).

2.1. Data handling and statistical analyses

Gas volume values were calculated for each time point by subtracting the corresponding gas measured in the substrate-negative control bottles and correcting for substrate dry matter. The mean control profiles for gas produced in inoculated culture bottles in the absence of substrate were subtracted prior to curve fitting analysis. Experimental data were fitted to the multi-phasic model of Groot et al. (1996):

$$Y = \frac{A}{(1 + (B/t)^C)}$$

where Y is the gas production (ml/g DM), A is the asymptotic gas production, B is the half time of asymptotic gas production, C is the curve shape parameter and t is the time.

The maximal fractional rate of substrate degradation (MFR; h^{-1}) was also calculated:

$$MFR = \frac{(C-1)^{(C-1)/C}}{B}$$

where MFR is the maximal fractional rate of substrate degradation, B is the half time of asymptotic gas production and C is the curve shape parameter.

Values for the modelled gas production parameters (as described above) were analysed for significant differences using two-way analysis of variance in GenStat Release 10.1 (Lawes Agricultural Trust, Harpenden, UK). Comparisons between treatment groups were made by LSD equations.