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Using an in vitro gas production technique to examine feed additives: Effects of correcting values for different blanks

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Abstract

Several in vitro experiments were conducted to determine effects of additives on rate and extent of gas production when several substrates were incubated in batch cultures of mixed rumen microorganisms. In all experiments, 500 mg of substrate was incubated with 50 ml of buffered rumen fluid at 39 °C and gas production was measured at 2, 4, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96 and 120 h. In experiments involving organic acids, three substrates differing in their forage:concentrate ratio (80:20 (F80), 50:50 (F50) and 20:80 (F20)) were incubated without additives, but in the presence of two doses (4 and 8 mM) of fumarate or malate. In the experiment with fibrolytic enzymes, samples of four tropical forages were incubated without additives and with three different enzyme solutions (cellulase, xylanase, and a 1:1 mixture of cellulase and xylanase) added at a rate of 20 IU/g dry matter forage. Blanks (i.e., bottles without sample) containing only buffered rumen fluid (B0) or buffered rumen fluid plus the corresponding dose of each additive (BA) were included for correction. Gas production values were fitted to the exponential model: gas = $A(1 - e^{(-c(t - \text{lag}))})$, and the average gas production rate (AGPR; ml gas/h) and organic matter effective degradability (OMED) were calculated. There were no blank × substrate or blank × additive interactions in any experiment. When B0 were used to correct gas production values, adding malate linearly increased ($P<0.05$) parameters A (asymptotic gas production) and AGPR for all substrates, but no differences due to malate addition occurred for any substrate when gas values were corrected for BA. Adding fumarate to all substrates linearly increased ($P<0.05$) parameters A, c (fractional rate of gas production) and AGPR when B0 was used for calculations, but it only tended ($P<0.10$) to linearly increase AGFR for substrate F80 and asymptotic gas production for substrate F50 when using BA for calculations. In the experiment with enzymes, there were only

Abbreviations: AGPR, average gas production rate; DM, dry matter; IVGPT, in vitro gas production technique; OMED, organic matter effective degradability

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subtle differences between results obtained using B0 and BA for correcting gas values. With both blanks, treatment of forages with cellulase and the cellulase:xylanase mixture decreased ($P<0.05$) lag and increased ($P<0.05$) OMED for all forages. Results demonstrate that using different blanks for correction purposes can produce different calculated effects between treatments, and therefore, can change interpretation of results, but this effect depends on the type of additives examined.

Keywords: Gas production; Batch cultures; Blanks; Organic acids; Enzymes

1. Introduction

In vitro gas production techniques (IVGPT) have been widely used over the past 20 years for feed evaluation (Pell et al., 1998). These techniques are relatively simple and inexpensive, and are increasingly being used for purposes beyond its original intent, such as to estimate microbial activity (Williams et al., 2000), to evaluate toxicity of secondary compounds (Ammar et al., 2004) and to screen effects of feed additives on rumen fermentation (Colombatto et al., 2003a). Since there are differences in rumen fluid activity among days (Menke and Steingass, 1988), blanks (i.e., bottles without substrate) are incubated to correct gas production values for gas released from fermentation of endogenous substrates.

In studies using IVGPT to investigate effects of feed additives on rumen fermentation, the possible contribution of additives to gas production has often been overlooked. Some additives interact with feeds and improve their nutritional value (i.e., enzymes), but others are fermented by rumen microorganisms and can increase their growth (i.e., organic acids). Although restricted to a small number of studies (Colombatto et al., 2003a,b; García-Martínez et al., in press), blanks containing the additive have been used for correction purposes, but the question as to whether additives should be included in the blanks remains unresolved.

We present results from several experiments conducted with organic acid and fibrolytic enzyme additives, in which blanks without (B0) and with (BA) the corresponding additive were used to correct gas production values. A primary objective was to investigate the effects of using different blanks on estimation of gas production parameters when different substrates were incubated in batch cultures of mixed rumen microorganisms. A second objective was to assess effects that differences have on the comparison of gas production profiles among treatments (i.e., control and additives).

2. Materials and methods

2.1. Substrates, animals and experimental procedure

Three experiments were conducted. In experiments 1 and 2 with organic acids, three substrates with forage:concentrate ratios (dry matter (DM) basis) of 80:20 (F80), 50:50 (F50) and 20:80 (F20) were incubated. Forage was alfalfa hay and maize silage (50:50; DM basis) and concentrate was based on barley grain, maize grain and soyabean meal (50:35:15;