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Short communication

Effect of *in vitro* techniques and exogenous feed enzymes on feed digestion☆

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ABSTRACT

The objective of this study was to assess whether changing gas production (GP) recording method (GPR), substrate delivery method (MD), and fineness of substrate grinding (FG) alter *in vitro* GP from feed digestion with and without exogenous feed enzymes (EFE) treatment. The experiment was a 2 GPR × 2 MD × 2 FG × 2 EFE factorial arrangement using barley straw, alfalfa hay or wheat dried distillers grain with solubles (DDGS) as substrates. There was no interaction of EFE with GPR, MD or FG on GP and dry matter digestibility (DMD). Compared to automated methods, manual recording increased ($P < 0.01$) GP, but enclosing substrate in bags vs. dispersing it in media reduced ($P < 0.04$) GP from both alfalfa hay and DDGS. Gas production was greater ($P < 0.05$) with 2 mm vs. 1 mm ground barley straw. The manual vs. automated GP recording resulted in reduced ($P < 0.01$) DMD of alfalfa hay and wheat DDGS. For all three substrates, DMD was consistently higher ($P < 0.01$) when enclosed in bags vs. dispersed in bottles. The DMD of alfalfa hay was improved ($P < 0.04$) with reducing FG (1 vs. 2 mm). Moreover, EFE improved ($P < 0.01$) the DMD of DDGS without affecting that of barley straw or alfalfa hay. These results showed that the GPR, MD and FG were little interacted with EFE effect on *in vitro* GP and DMD of feeds, suggesting that the impact of these factors on *in vitro* EFE effect is minimal. Hence, enclosing feed substrate in filter bags can be recommended to screen EFE products for feed digestion because of practical convenience. Moreover, the impacts of GPR, MD and FG on *in vitro* GP and DMD of feeds suggested that these factors should be carefully considered when comparing the nutritive values of various feeds using *in vitro* techniques.

1. Introduction

The method of *in vitro* batch culture has been widely applied to screen and compare various feeds and additives. Although the advantages of using *in vitro* techniques as compared to *in vivo* methods are many, a number of factors used in the batch

Abbreviations: aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; DDGS, dried distillers grain with solubles; DM, dry matter; DMD, DM digestibility; EFE, exogenous feed enzymes; FG, fineness of substrate grinding; GP, gas production; GPR, GP recording method; MD, substrate delivery method; OM, organic matter.

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culture method including recording system of gas production (GP), method of substrate dispersal in the bottle, and method of substrate preparation can alter fermentation results. Venting methods for GP measurement is a noticeable issue. In a closed system, gas accumulates and the rise in pressure in headspace may affect the rate of substrate fermentation. Tagliapietra et al. (2010) reported that using manual pressure measurements, headspace volume, venting frequency and amount of fermentable substrate must be carefully balanced to avoid high headspace pressures that could alter fermentation kinetics. Moreover, previous studies have shown that the substrates could be placed in porous nylon bags within incubation vials (He et al., 2013) or freely into the inoculum (Elghandour et al., 2013) when screening exogenous feed enzymes (EFE). It is possible that the nylon bags create a microenvironment that is distinct from that of free inoculum and may vary with changes in the pore size of bags (Mendoza et al., 2014). Finally, use of a finely ground sample reduces the risk of sampling bias, but fine particles may exit the bags prior to true digestion resulting in an overestimation of feed digestion. The objective of this study was to assess whether the GP recording method (GPR), substrate delivery method (MD) and fineness of substrate grinding (FG) influence responses observed when substrates are treated with EFE.

2. Materials and methods

2.1. Feed samples, enzyme product and GP system

Barley straw, alfalfa hay and wheat dried distillers grain with solubles (DDGS) representing low and high quality roughage, and high fiber concentrate, respectively, were used in this study. Barley straw contained (g/kg dry matter [DM]): 925 organic matter (OM), 37 crude protein (CP) and 791 neutral detergent fiber (aNDF). Alfalfa hay was composed of (g/kg DM): 908 OM, 185CP and 532 aNDF. Both barley straw and alfalfa hay were ground through 1-mm or 2-mm screens using a Wiley mill (standard model 4, Arthur Thomas Co., Philadelphia, PA, USA). Wheat DDGS was used without grinding because of its fine particle size, and contained (g/kg DM) 935 OM, 382CP and 397 aNDF.

The EFE (AB Vista, Marlborough, UK) used in this study was in the form of a liquid and was previously shown to increase the *in vitro* DM digestibility (DMD) of wheat DDGS (He et al., 2013). It was a blend of xylanase and glucanase with the xylanase originating from a strain of *Bacillus subtilis* that was subsequently expressed in *Trichoderma reesei* whereas the glucanase arose directly from a strain of *T. reesei*. The EFE activities were 878, 167, 38 and 3.9 μmol/min per mL, respectively, for xylanase, endoglucanase, exoglucanase and protease, which were assayed at pH 6.0 and 39 °C as detailed in our previous study (He et al., 2013).

Serum bottles (100 mL) sealed with a rubber stopper were used for manual GP recording and a 500-mL Ankom GP module (a computerized system with automated pressure transducers, Ankom Technology, Macedon, NY, USA) equipped with an Ankom pressure sensor module including a microchip and a radio transponder was used for automated GP recording.

2.2. In vitro incubations

On the day prior to incubation, 0.5 g of substrate (ground 1 or 2 mm) was weighed into acetone-washed and pre-weighed filter bags (pore size of 25 μm), which were sealed and placed in bottles or 0.5 g was added directly into 100-mL serum bottles (3 replicates). For the 500-mL bottles, 3 bags, each containing 0.5 g substrate were added to each bottle or 1.5 g of substrate were added directly into each bottle. Anaerobic medium was added (48 mL into each 100-mL bottle and 144 mL into each 500-mL bottle) in a manner that ensured the medium to substrate ratio remained the same between the two systems. Meanwhile, a volume of 200 μL (in 100-mL bottle) or 600 μL (in 500-mL bottle) EFE solution was anaerobically added to achieve an enzyme dose of 2 μL/g of DM substrate. Then the bottles were capped with stoppers and stored at room temperature for approximately 17 h.

Ruminal fluid was collected 2 h after the morning feeding from two ruminally fistulated beef heifers fed a diet containing (g/kg DM) 600 barley silage, 350 dry-rolled barley grain and 50 of a mineral and vitamin supplement. The heifers were managed according to the guidelines of the Canadian Council on Animal Care (2009). Ruminal contents of each heifer were obtained from various locations within the rumen, and squeezed through PeCAP® polyester screen (pore size 355 μm; B & S H Thompson, Ville Mont-Royal, QC, Canada). The strained ruminal fluids from the two heifers were pooled in equal proportions into a pre-warmed, insulated container and transferred to the laboratory. Inoculum was warmed to 39 °C in a water bath and flushed with oxygen free CO_2 before being dispensed (12 mL into each 100-mL bottle and 36 mL into each 500-mL bottle). Bottles were sealed after loading, and placed in an incubator at 39 °C for 24 h with shaking (125 rpm/min). Blank controls were also incubated to correct for gas release and residual fermentation resulting directly from the inocula.

Headspace gas pressure in the small serum bottles were manually recorded at 3, 6, 9, 12, and 24 h post inoculation. For the wireless Ankom system, the pressure changes in the headspace of the bottles were transmitted via radio frequency to a computer at 5 min intervals and accumulated gas in the headspace was automatically released when the pressure reached at 3.0 kPa. After correction for the blanks, the recorded cumulative gas pressure was converted to mL of gas produced under the manufacturer's instructions using Avogadro's law (gas volume, mL = gas pressure × [V/RT] × 22.4 × 1000, where '*V*' is head-space volume in the bottle in L, '*R*' is the gas constant 8.314472 L kPa/K/mol and '*T*' is the temperature in Kelvin).

After 24 h of incubation, bags were removed from the bottles, washed and dried in an oven at 55 °C for 48 h to estimate DMD. For the feed that was dispersed directly into the inocula, the contents of the bottles were filtered through pre-weighed bags (20 cm × 10 cm) made of monofilament PeCAP® polyester screen (pore size of 50 μm). All determinations were