

Concentration of volatile fatty acids (VFA) in ruminal fluid samples was determined in a liquid-liquid solvent extraction using ethyl acetate (Ruiz-Moreno et al., 2015). Samples were centrifuged for 15 min at  $10,000 \times g$  at  $4^{\circ}\text{C}$  (Avanti J-E, Beckman Coulter Inc., Palo Alto, CA). Ruminal fluid supernatant was mixed with a meta-phosphoric acid (25% wt/vol): crotonic acid (2g/L, internal standard) solution at a 5:1 ratio and samples were frozen overnight, thawed, and centrifuged for 10 min at  $10,000 \times g$  at  $4^{\circ}\text{C}$ . Supernatant was transferred into glass tubes (12 mm  $\times$  75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), and mixed with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant. After shaking tubes vigorously and allowing the fractions to separate, the ethyl acetate fraction (top layer) was transferred to vials (9 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA). Samples were analyzed by gas chromatography (Agilent 7820A GC, Agilent Technologies, Palo Alto, CA) using a flame ionization detector and a capillary column (CP-WAX 58 FFAP 25 m  $\times$  0.53 mm, Varian CP7767, Varian Analytical Instruments, Walnut Creek, CA). Column temperature was maintained at  $110^{\circ}\text{C}$ , and injector and detector temperatures were 200 and  $220^{\circ}\text{C}$ , respectively.

Ruiz-Moreno, M., E. Binversie, S. W. Fessended, and M. D. Stern. 2015. Mitigation of in vitro hydrogen sulfide production using bismuth subsalicylate with and without monensin in beef feedlot diets. *J. Anim. Sci.* 93:5346-5354. doi:10.2527/jas2015-9392