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Association of aqueous hydrogen concentration with methane production in continuous cultures modulated to vary pH and solids passage rate

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ABSTRACT

The objective of this study was to evaluate the effects of altering pH and solids passage rate (k_p) on concentration of aqueous H_2 [H_2(aq)], CH_4 production, volatile fatty acids (VFA) production, and fiber digestibility in a continuous culture fermentation system. The present study was conducted as a 2 × 2 factorial treatment arrangement in a Latin square design using continuous culture fermentors (n = 4). Our continuous culture system was converted to a closed system to measure CH_4 and H_2 emission while measuring H_2(aq) concentration and VFA production for complete stoichiometric assessment of fermentation pattern. Treatments were control pH (CpH; ranging from 6.3 to 6.9) or low pH (LpH; 5.8 to 6.4) factorialized with solids k_p that was adjusted to be either low (Lk_p; 2.5%/h) or high (Hk_p; 5.0%/h); liquid dilution was maintained at 7.0%/h. Fermentors were fed once daily (40 g of dry matter; 50:50 concentrate:forage diet). Four periods lasted 10 d each, with 3 d of sample collection. The main effect of LpH increased nonammonia nitrogen flow, and both LpH and Hk_p increased nonammonia nonbacterial N flow. We observed a tendency for Hk_p to increase bacterial N flow per unit of nonstructural carbohydrates and neutral detergent fiber degraded. The main effect of LpH decreased H_2(aq) by 4.33 μM compared with CpH. The main effect of LpH decreased CH_4 production rate from 5 to 9 h postfeeding, and Hk_p decreased CH_4 production rate from 3 to 9 h postfeeding. We found no effect of LpH on daily CH_4 production or CH_4 produced per gram of neutral detergent fiber degraded, but Hk_p decreased daily CH_4 production by 33.2%. Both the main effects of LpH and Hk_p decreased acetate molar percentage compared with CpH and Lk_p, respectively. The main effect of both LpH and Hk_p increased propionate molar percentage, decreasing acetate-to-propionate ratio from 2.62 to 2.34. We noted no treatment effects on butyrate molar percentage or total VFA production. The results indicate increasing k_p and decreasing pH decreased acetate-to-propionate ratio, but only increasing k_p decreased CH_4 production; lack of differences for LpH might be a result of compensatory methanogenesis during the second half of the day postfeeding.
Key words: methane, continuous culture, passage rate, pH, hydrogen

INTRODUCTION

Greenhouse gases originating from livestock production are a target of environmental emissions mitigation strategies, but estimates of the contribution of enteric CH_4 to global or national emissions vary based on assumptions inherent in the estimate approach (Hristov et al., 2014). Knapp et al. (2014) reported estimates of enteric CH_4 representing 17% of global CH_4 emissions. Enteric fermentation in dairy cattle is estimated to contribute approximately 3.9% of total anthropogenic CH_4 emissions in the United States (EPA, 2013).

A recent hypothesis proposed by Janssen (2010) expanded our perspective on the relationship between physiological changes instigated by dietary or feed additive treatment and their effect on the concentration of aqueous H_2 [$\mathbf{H_2(aq)}$], shifting fermentation pathways. Increasing H_2(aq) concentration was hypothesized to be required to maintain methanogen growth rates with increasing solids passage rate ($\mathbf{k_p}$) based on steady-state microbial growth kinetics (Monod, 1949; Janssen, 2010). With methanogens limited by decreased pH, Janssen (2010) proposed that H_2(aq) would also increase under these conditions because methanogens will lower their relative growth rate under current conditions given a decreased maximum growth rate (Janssen, 2010). Rather than assuming that decreased relative growth rate will simply decrease competitive assimilation of H_2(aq) into methanogenesis and allow H_2(aq) to increase, the kinetic model hypothesizes that low pH decreases the maximal growth rate, which will shift upward the H_2(aq) concentration needed to main-

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tain relative growth rates. Increased concentrations of H_2(aq) from either increased k_p or lowered pH were predicted to thermodynamically shift VFA production toward pathways that decrease net microbial production of H_2, particularly the acetate pathways that would otherwise yield the most H_2 (Janssen, 2010). Decreasing total H_2 produced would then ultimately decrease total CH_4 production.

We proposed to use continuous culture fermentors as a model to measure concentration of H_2(aq), CH_4 production, VFA production, and fiber digestibility as a screening and validation process for the model by Janssen (2010). We hypothesized that lower culture pH would inhibit methanogens, necessitating an increase in concentration of H_2(aq), decreasing production of H_2 and CH_4, and shifting VFA pathways toward a lower acetate-to-propionate ratio. Alternatively, increased solids k_p would increase methanogen relative growth rate (Monod, 1949; Janssen, 2010) and increase the concentration of H_2(aq), thereby decreasing CH_4 production and again shifting VFA production pathways toward a lower acetate-to-propionate ratio. Because k_p and pH are presumed to affect methanogens via modes of action based on distinct growth or microbial activity inhibitions, we hypothesized that the combination of low pH and high k_p would not be additive (i.e., we would detect a significant statistical interaction).

MATERIALS AND METHODS

Experimental Design

The present study was conducted as a 2 × 2 factorial treatment arrangement in a Latin square design using dual-flow continuous culture fermentors (n = 4). Fermentors were fed once daily [40 g of DM; 50:50 concentrate:forage diet, 37.5% NDF, 19.5% NSC (starch plus water-soluble carbohydrate), and 15.4% CP]. The pelleted forage was an alfalfa meal pellet and the pelleted concentrate was composed of ground corn (36.9%), soybean hulls (40.4%), dried distiller's grains (10.0%), 48% CP soybean meal (9.1%), corn oil (1.8%), MgO (0.2%), and trace-mineralized salt (0.9%). The latter contained Na (36.6%), Cl (56.4%), Zn (3500 mg/kg), Mn (2,800 mg/kg), Fe (1,750 mg/kg), Cu (350 mg/kg), I (70 mg/kg), and Co (70 mg/kg). The buffer also contained minerals that would support optimal microbial function. Treatments were control pH (**CpH**; diurnally ranging from 6.3 to 6.9) or low pH (**LpH**; 5.8 to 6.4) factorialized with solids k_p adjusted to be either low (**Lk$_p$**; 2.5%/h) or high (**Hk$_p$**; 5.0%/h). Liquid dilution was maintained at 7.0%/h to maintain constant infusion of buffer (for more accurate control of pH treatments) and to avoid a confounding dilution factor in H_2(aq) expectations. Four experimental periods lasted 10 d each, with 3 d of sample collection at the end of each period. Buffer pH for CpH treatments was made according to Weller and Pilgrim (1974), with the addition of 0.4 g/L of urea, and determined to be constant at pH 6.8 under continuous bubbling with CO_2. For the LpH treatment, we added H_3PO_4 to a concentration of 12.5 m*M* to decrease buffer pH to 6.4. Both buffers allowed a parallel diurnal shift in pH (see Supplemental Figure S1; https://doi.org/10.3168/jds.2016-12332) to better reflect normal rumen function in vivo postfeeding (Cantalapiedra-Hijar et al., 2011). The CpH treatment allowed fluctuation of fermentor pH from 6.9 prefeeding to 6.3 approximately 8 h postfeeding, with a gradual return over the remaining 16 h of each experimental day. In a parallel response, the LpH treatment fluctuated between 6.4 prefeeding and 5.8 postfeeding. The fermentor pH was not allowed to drop below 5.8 to prevent long-term effects on protozoa (Dehority, 2010) that might confound treatment effects by decreasing protozoal metabolic activity and increasing bacterial abundance in replacement of lost protozoa.

Continuous Culture Operation

Dual-flow continuous culture fermentors (n = 4; Hoover et al., 1976), adapted for protozoa retention similar to Karnati et al. (2009), were used for this experiment. Average fermentor volume was 1.71 L (range: ± 0.09 L), and total buffer dilution rate was maintained at 7.0%/h. At the beginning of each period, rumen contents were manually sampled from 2 ruminally cannulated lactating Jersey cows fed a lactating diet, rapidly squeezed through 2 layers of cheesecloth, and then liquid was placed in insulated containers maintained at 39°C. Samples were pooled and inoculated into fermentors at 50% of fermentor volume. Sampling to inoculation interval averaged 30 min and did not exceed 40 min. Clarified rumen fluid (centrifuged at 15,000 × *g*, 4°C, 15 min, and autoclaved) was used in a 1:20 dilution of buffer for the first 3 d of each period to better adapt protozoa to fermentors; in addition, filters retained protozoa except for efflux with the solids as described previously (Karnati et al., 2009; Wenner, 2016). Fermentor buffer was made according to Weller and Pilgrim (1974); 40 mg/dL of urea was included to prevent depression of microbial protein synthesis because fermentors also remove the effect of N recycling observed in vivo. Buffer was continuously bubbled with CO_2 for at least 1 d before use to maintain anaerobic conditions in the fermentors. Treatments were imposed after 2 d of adaptation to the fermentors following each experimental period's inoculation.