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Essential oil and monensin affect ruminal fermentation and the protozoal population in continuous culture

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ABSTRACT

The interaction of monensin and essential oil was hypothesized to suppress protozoa and methane production while maintaining normal rumen function. The objective of this study was to determine the effects of feeding monensin (MON) and CinnaGar (CIN, a commercial blend of cinnamaldehyde and garlic oil) on ruminal fermentation characteristics. Continuous culture fermentors (n = 4) were maintained in 4 experimental periods in a 4 × 4 Latin square design. Four dietary treatments were arranged in a 2 × 2 factorial: (1) control diet, 37 g/d of dry matter (40 g/d at ~92.5% dry matter) of a 50:50 forage:concentrate diet containing no additive; (2) MON at 11 g/909 kg of dry matter; (3) CIN at 0.0043% of dry matter; and (4) a combination of MON and CIN at the levels in (2) and (3). Treatment had no effects on protozoal populations, concentration of NH_3N, total N flow of effluent, production of total volatile fatty acids, or flows of conjugated linoleic acid and total C18 fatty acids. The MON decreased acetate: propionate ratio and biohydrogenation of both total C18 and 18:1 *cis*-9 but increased protozoal generation time, concentration of peptide, and flow of 18:1 *trans*-11. The MON tended to decrease protozoal counts in effluent and flow of 18:0 but tended to increase propionate production. The CIN decreased true organic matter digestibility and protozoal N flow of effluent but increased nonammonia, nonmicrobial N flow. The CIN tended to decrease protozoal counts, microbial N flow, and neutral detergent fiber digestibility but tended to increase biohydrogenation of total C18, 18:2, and 18:3. The CIN tended to increase isovalerate production. The MON and CIN tended to interact for increased methane production and bacterial N flow. A second experiment was conducted to determine the effects of MON and CIN on protozoal nitrogen and cell volume in vitro. Four treatments included (1) control (feed only), (2) feed + 0.0043% dry matter CIN, (3) feed + 2.82 μM MON, and (4) feed + CIN + MON at the same levels as in (2) and (3). With no interactions, MON addition decreased percentage of protozoa that were motile and tended to decrease cell volume at 6 h. The CIN did not affect cell count or other indicators of motility or volume at either 3 or 6 h. Under the conditions of our study, we did not detect an additive response for MON and CIN to decrease protozoal counts or methane production. A 3-dimensional method is suggested to better estimate protozoal cell volume.

Key words: essential oil, monensin, protozoa, continuous culture

INTRODUCTION

Increasing public concern over climate change and increasing emphasis on rumen-derived methanogenesis have directed considerable research efforts to investigating suppression of ruminal protozoa because of their close association with methanogens (Newbold et al., 2015). By decreasing methanogenesis in the rumen, reducing equivalents may be directed to propionate (the major gluconeogenic precursor in ruminants) rather than being eructated. This strategy of inhibiting protozoa to reduce methanogenesis is complicated because results have not been found to be long lasting and reduced protozoal concentrations are not always correlated with reduced methane production (Williams et al., 2009).

The use of essential oil (**EO**), including cinnamaldehyde, as an alternative to antibiotics in animals is gaining wide attention because of its antimicrobial property, likely a result of microbial cell membrane disruption (Gill and Holley, 2004), but the activity is nonspecific against bacteria, protozoa, and fungi (Cobellis et al., 2016). Many EO also have suppressed methanogenesis (Cobellis et al., 2016). Essential oils have been

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suggested as a potential tool for manipulation of the bacterial populations involved in ruminal biohydrogenation of fatty acids (**FA**; Calsamiglia et al., 2007). Cinnamaldehyde decreased apparent biohydrogenation of 18:2n-6 and 18:3n-3 in a continuous culture fermentor system and shifted the biohydrogenation from the *trans*-11 pathway to the accumulation of 18:1 *trans*-10 and *trans*-10,*cis*-12 CLA (Lourenço et al., 2008). This *trans*-10 shift has been documented to cause milk fat depression in vivo (Jenkins et al., 2008). However, supplementation of TMR for lactating dairy cows with cinnamaldehyde (1 g/d) did not modify the FA profile of milk fat (Benchaar and Chouinard, 2009).

Monensin (**MON**) has decreased the rate of ruminal biohydrogenation of UFA in vitro (Fellner et al., 1997) and increased the concentration of CLA in milk fat (AlZahal et al., 2008). Protozoa adapt to MON by changing their membrane structure (Karnati et al., 2009; Sylvester et al., 2009). However, it is unknown if EO along with MON would suppress protozoa long term. Moreover, direct suppression of protozoa (with a specific inhibitor) could limit fermentation rate and increase the generation time of protozoa, making them less competitive and thus lowering their biomass in the rumen (Karnati et al., 2009).

The suppression of ruminal microbes, especially protozoa, by EO and MON is not fully understood, and limited data are available on the effects of a combination of MON and EO. The MON inhibits hydrogen-producing bacteria (Chen and Wolin, 1979) and causes depletion of energy reserves (Tedeschi et al., 2003). Membrane leaks could occur with decay of proton motive force when the protein:lipid of the membrane increases in artificial liposomes (Russell and Strobel, 1989). However, more current information suggests that sensitivity to MON depends on specific cell wall susceptibility, not previous associations with gram-positive and gram-negative staining (Scharen et al., 2017). In that study, protozoal counts were not associated with MON or EO supplementation, apparently as a result of adaptation, and a combination of MON and EO was not studied. Nutrient stress could cause increased autophagy or decreased protozoal cells in division state (Berger, 2001). Thus, cellular adaptations associated with decreasing volume and cellular protein concentrations are assumed to be a stress response to introduction of MON and EO.

We hypothesized that an interaction of MON with CinnaGar (a blend of cinnamaldehyde and garlic oil) would suppress protozoa and their associated methanogens, but this suppression must be characterized within the context of compensatory increases in the bacterial activity to outcompete protozoa during their adjustment to these additives. The objective of this study was to determine the effects of feeding MON and CinnaGar in diets on ruminal fermentation characteristics, nutrient digestibility, and microbial efficiency in continuous culture and protozoal counts and their cell volume after adaptation to these additives.

MATERIALS AND METHODS

Design of Experiment 1

A modified dual-flow continuous culture system was used in the study. Four continuous culture fermentors in a 4 × 4 Latin square design were modified to retain protozoa and maintained in 4 periods of 10 d each (7 d of adaptation). The fermentors were fed once daily 37 g/d of DM (40 g/d at ~92.5% DM) of a 50:50 forage: concentrate diet (38% NDF, 16% CP) containing either no additive, MON (Elanco Animal Health, Greenfield, IN) as Rumensin at 11 g/909 kg of DM in the pelleted concentrate, CinnaGar (**CIN**) provided by Provimi North America (Brookville, OH) at 0.0043% of feed mixture DM, or MON (11 g/909 kg of DM) plus CIN (0.0043% DM basis; Tables 1 and 2). The CIN was provided just before initiation of our study and was kept sealed and refrigerated to prevent volatilization of active product. The dosage of MON is based on the company label, and the dose of CIN is comparable to 1 g/d of cinnamaldehyde to dairy cattle (assuming 23 kg/d of DMI) as justified by Benchaar and Chouinard (2009).

The dual-flow continuous culture system was based on the system initially described by Hoover et al. (1976). For each period, ruminal contents were taken 2 h postfeeding from 2 cannulated Holstein cows that were maintained on a diet (approximately 50% forage with corn silage as the primary forage) without EO or MON at The Ohio State University's Waterman Dairy Center. The contents were pooled and squeezed through 2 layers of cheesecloth into a container maintained at 39°C. After being transported to the laboratory, the rumen fluid was re-strained through 2 layers of cheesecloth, and 500 mL was inoculated into each fermentor, then buffer was added while mixing to fill up to the overflow level. As described in Karnati et al. (2009), a multi-stage filter system was used on the pumps to retain protozoa. The volumes of the 4 fermentors ranged from 1.63 to 1.81 L. The pH in the fermentors was monitored and adjusted between 6.4 and 6.8 during the feeding cycle of the adaptation phase and was unchanged during the collection phase. Temperature was maintained at 39°C, and agitation was set at 50 rpm. Flow rates were determined once a day during the adaptation period by weighing solid (overflow) and liquid (filtrate plus overflow) outflows from each fermentor