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Capsaicin influences rumen microbial fermentation and gas production in vitro[1]

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ABSTRACT: This study was preliminary to a research project that evaluated the potential for capsaicin to decrease inflammation in cattle. The objective of this research was to evaluate the effects of capsaicin on rumen microbial fermentation and gas production in an in vitro system. Rumen fluid was collected from 2 ruminally-cannulated heifers fed an alfalfa hay-based diet. Strained rumen fluid (50 mL) was mixed with McDougal's buffer (50 mL) and anaerobically incubated at 39°C in 250 mL Erlenmeyer flasks that contained treatments. Treatments were 1 g of ground alfalfa hay that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (contained 1,280 ppm capsaicin). Gas production was measured from 24 flasks (12 replicates per treatment) that were incubated for 24 h, and gas measurements were recorded at 0, 2, 4, 6, 8, 10, 12, 18, and 24 h. Rumen microbial fermentation products (pH, NH3, and VFA) were collected from 2 runs of 32 incubating flasks that were stopped after 0, 6, 12, or 24 h of incubation (8 replicates per treatment at each incubation time). Data were analyzed statistically as repeated measures using mixed models. A treatment × hour interaction ($P = 0.02$) occurred for gas production; gas production was not different at 0, 2, 8, 10, 12, 18, and 24 h, but was greater ($P < 0.05$) for CAP than CON at 4 and 6 h. No treatment × hour interactions ($P \geq 0.24$) occurred for pH, total VFA, and individual VFA proportions. Ammonia concentrations tended to be lower for CAP than CON at 24 h (treatment × hour, $P = 0.12$). Ammonia and total VFA concentrations were not different ($P \geq 0.23$) between CON and CAP, and pH tended to be greater ($P = 0.11$) for CAP than CON. Molar percentages of acetate were greater ($P < 0.01$), and molar percentages of propionate, butyrate, and valerate were lower ($P < 0.01$) for CAP than CON. Thus, acetate:propionate ratio was greater ($P < 0.01$) for CAP than CON. Ruminal pH decreased ($P < 0.01$), and concentrations of NH3 and total VFA increased ($P < 0.01$) as incubation time increased. These results demonstrate that the addition of 2% jalapeño powder to a ground alfalfa hay substrate altered rumen microbial fermentation and gas production. These effects on rumen microbial fermentation were in favor of acetate production.

Key words: capsaicin, in vitro, rumen, volatile fatty acid

INTRODUCTION

Capsaicin is the major capsaicinoid in hot peppers and is responsible for a pungent sensation when consumed. In addition to its use in cuisine, capsaicin has pharmacological uses because of its role in pain management, cardiovascular, respiratory and other biological systems (O'Neill et al., 2012). Dogan et al. (2004) reported that capsaicin reduced lipopolysaccharide-induced fever in rats, and Demirbilek et al. (2004) demonstrated that capsaicin decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines in septic rats. Therefore, feeding capsaicin in hot peppers to ruminant livestock may be effective at reducing fever when they have been exposed to stress and disease.

Natural plant extracts, including capsaicin, have been shown to alter rumen microbial fermentation (Cardozo et al., 2004; 2005; 2006). According to Cardozo et al. (2005), the effects of natural plant extracts on rumen microbial fermentation is variable, and is dependent on the ruminal pH. Also, Cazac et al. (2005) demonstrated that ruminal degradation of chile peppers is greater in forage-based diets than in concentrate-based diets. Therefore, the effects of capsaicin on rumen microbial fermentation should be researched further before being evaluated as an anti-inflammatory supplement for ruminant animals. The objective of this study was to evaluate rumen microbial fermentation and gas production in response to the addition of jalapeño powder to a ground alfalfa hay substrate in an in vitro system.

MATERIALS AND METHODS

In Vitro Procedure

This research was preliminary to another project that evaluated the potential for capsaicin to decrease inflammation in cattle (Samuelson et al., 2014). All procedures were approved by the Institutional Animal Care and Use Committee of New Mexico State University. Rumen fluid was collected from 2 ruminally-cannulated heifers receiving an alfalfa hay diet. Equal amounts of rumen fluid from each heifer were mixed together and strained through cheesecloth into a pre-warmed (±39°C) thermos for transportation to the laboratory. The strained rumen fluid was mixed with an equal volume of

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pre-warmed (±39°C) McDougal's buffer (Tilley and Terry, 1963), and 100 mL of this mixture was added to 250 mL Erlenmeyer flasks that contained treatments. The Erlenmeyer flasks were then flushed with CO2 to displace O2 from the gaseous head space, and were fitted with rubber stoppers connected to 250-mL inverted burette cylinders with silicone tubing. Erlenmeyer flasks were incubated at 39°C in a LAB-LINE Orbit Environmental Shaker that provided continuous rotational movement.

Treatments

Treatments were alfalfa hay (ground to pass a 2 mm screen) that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin. One gram of either CON or CAP was weighed into 250-mL Erlenmeyer flasks before anaerobic incubation with equal volumes of rumen fluid and McDougal's buffer in a LAB-LINE Orbit Environmental Shaker.

Gas Production

For the measurements of gas production, 24 Erlenmeyer flasks containing CON or CAP treatment, rumen fluid and McDougal's buffer were incubated for 24 h in a completely randomized design. All flasks (12 flasks per treatment) were incubated for 24 h without interruption, and the volume of water displacement was recorded as a measure for gas production at 0, 2, 4, 6, 8, 10, 12, 18, and 24 h of incubation. Displacement of water by the production of gas was measured using 250-mL burette cylinders that were inverted into 600 mL beakers filled with water. The spout of the burette cylinders were connected to the incubating Erlenmeyer flasks with a silicone tube to capture the production of gas.

Rumen Microbial Fermentation

For the measurements of rumen microbial fermentation products, 2 runs of 32 Erlenmeyer flasks containing CON or CAP treatments, rumen fluid and McDougal's buffer were incubated for 0, 6, 12, or 24 h in a randomized complete block design. This experiment was blocked by run because of limited space in the incubator. For both runs, there were a total of 8 replicate flasks per treatment at each incubation time. Erlenmeyer flasks that were assigned to an incubation hour were removed from the incubator when the appropriate time had elapsed (other fermentation flasks were not disturbed during this process). The pH of the fluid was measured immediately after the termination of fermentation using a portable pH meter (Mettler Toledo 8603, Schwerzenbach, Switzerland). Then, two 10-mL samples of fluid were transferred from each incubation flask to separate scintillation vials and immediately frozen at -20°C to stop further fermentation by anaerobic micro-organisms. Samples were later thawed, centrifuged (Eppendorf, Hamburg, Germany) at 28,000 × g for 20 min, and the supernatant was analyzed for VFA and NH3 concentrations. Individual VFA concentration were determined using capillary gas chromatography (Varian 3400, Varian Inc., Walnut Creek, CA) in accordance to May and Galyean (1996), and NH3 concentrations were determined according to the procedure of Broderick and Kang (1980) adjusted for a micro-plate reader (ELX 808 Ultra Micro Reader, Bio-Tek Instruments Inc., Winooski, VT).

Statistical Analysis

All data were analyzed statistically as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). Flask was the experimental unit. For gas production, the experiment was a completely randomized design. The statistical model included treatment, hour, and treatment × hour interaction. The covariance structure was compound symmetry. For rumen microbial fermentation products and pH, the experiment was a randomized complete block design. The statistical model included treatment, hour, and treatment × hour interaction. Block was random, and the covariance structure was autoregressive order one. This experiment was blocked by run (collection date) because space in the incubator limited the number of flasks that could be incubated at the same time. Differences were considered significant when $P < 0.05$.

RESULTS

An interaction between treatment and hour ($P = 0.02$) was observed for in vitro gas production; gas production was not different between CON and CAP at 0, 2, 8, 10, 12, 18, and 24 h, but was greater ($P < 0.05$) for CAP than CON at 4 and 6 h of incubation (Figure 1). No interactions between treatment and hour ($P \geq 0.24$) were observed for pH, total VFA concentrations, and molar percentages of individual VFA (Figure 2). Ammonia concentrations tended to be lower for CAP than CON at 24 h (treatment × hour, $P = 0.12$). Ammonia and total VFA concentrations were not different ($P \geq 0.23$) between CON and CAP, and pH tended to be greater ($P = 0.11$) for CAP than CON. Rumen fluid from in vitro fermentations containing CAP had greater (*P*

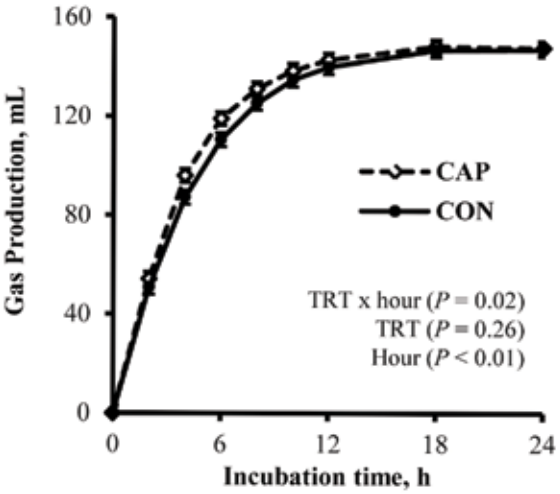

Figure 1. In vitro gas production from equal volumes of rumen fluid and McDougal's buffer when incubated with 1 g ground alfalfa hay that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin.

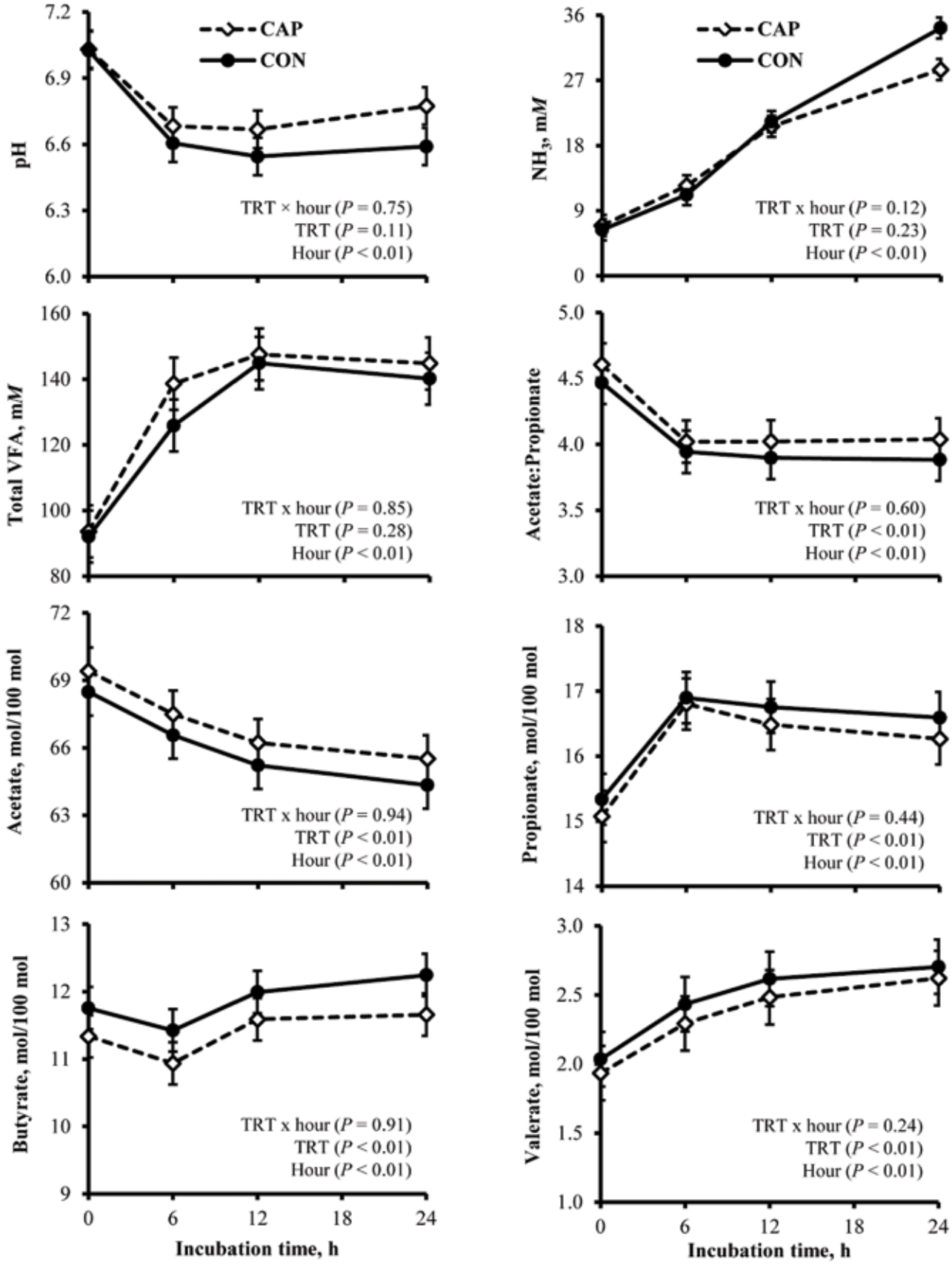

Figure 2. In vitro pH, NH3, and VFA concentrations of equal volumes of rumen fluid and McDougal's buffer when incubated for 0, 6, 12, and 24 h with 1 g ground alfalfa hay that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin.

< 0.01) molar percentages of acetate, and lower ($P < 0.01$) molar percentages of propionate, butyrate, and valerate, than in vitro fermentations containing CON. Therefore, the acetate:propionate ratio was greater ($P < 0.01$) for fermentation flasks containing CAP compared with CON. Regardless of treatment, pH decreased ($P < 0.01$), and concentrations of NH3 and total VFA increased ($P < 0.01$) as incubation time increased.

DISCUSSION

No significant differences between CAP and CON for ruminal pH, NH3, and total VFA concentrations suggest that CAP had little or no effects on rumen microbial fermentation in vitro. However, small increases in gas production as well as greater molar percentages of acetate, and lower molar percentages of propionate, butyrate, and valerate indicated that CAP shifted rumen microbial fermentation in favor of acetate production. This increase in the ratio of acetate:propionate may have resulted in the tendency for pH to increase in response to CAP. The observed shifts in individual VFA proportions are consistent with the results of Cardozo et al. (2005), who reported that *Capsicum annuum* containing 12% capsaicin increased acetate:propionate ratios when the in vitro ruminal pH was 7.0. Cardozo et al. (2005) also reported that capsaicin decreased both total VFA concentrations and NH3 concentrations. Although, total VFA concentrations were not affected in this study, CAP tended to decrease NH3 concentrations, which is an indication that amino acid deamination was perhaps decreased by capsaicin (Cardozo et al., 2005).

In conclusion, the results of this study indicated that the addition of 2% jalapeño powder (contained 1,280 ppm capsaicin) to a ground alfalfa hay substrate alters rumen microbial fermentation and gas production in an in vitro batch culture system. These effects on rumen microbial fermentation were in favor of acetate production. Further research is necessary to evaluate the degradation of capsaicin by rumen microbes so that the post-ruminal supply of capsaicin as a potential anti-inflammatory for ruminant animals can be quantified.

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