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The effects of a garlic oil chemical compound, propyl-propane thiosulfonate, on ruminal fermentation and fatty acid outflow in a dual-flow continuous culture system

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ABSTRACT

The ban on the use of antibiotics as growth promoters in animal feeds in the European Union has stimulated research on potential alternatives. Recently, propyl-propane thiosulfonate (PTSO), a stable organosulfurate compound of garlic, was purified. The objectives of the current study were to investigate the potential effects of PTSO on rumen microbial fermentation and to define effective doses. Two experiments were conducted using dual-flow continuous culture fermenters in 2 replicated periods. Each experimental period consisted of 5 d for adaptation of the ruminal fluid and 3 d for sampling. Temperature (39°C), pH (6.4), and liquid (0.10 h^{-1}) and solid (0.05 h^{-1}) dilution rates were maintained constant. Samples were taken 2 h after feeding and from the 24-h effluent. Samples were analyzed for volatile fatty acids (VFA) and nitrogen fractions, and degradation of nutrients was calculated. In addition, 24-h effluents from experiment 2 were analyzed for their fatty acid (FA) profile. Treatments in experiment 1 included a negative control without additive, a positive control with monensin (12 mg/L), and PTSO at 30 and 300 mg/L. The addition of 30 mg/L did not affect any of the measurements tested. The addition of 300 mg/L reduced microbial fermentation, as suggested by the decreased total VFA concentration, true degradation of organic matter and acid detergent fiber, and a tendency to decrease neutral detergent fiber degradation. Experiment 2 was conducted to test increasing doses of PTSO (0, 50, 100, and 150 mg/L) on rumen microbial fermentation. At 2 h postfeeding, total VFA and molar proportion of propionate responded quadratically, with higher values in the intermediate doses. Molar proportions of butyrate increased and branched-chain VFA decreased linearly as the dose of PTSO increased. In the 24-h effluents, total VFA, acetate, and branched-chain VFA concentrations decreased linearly and those of propionate responded cubically with the highest value at 100 mg/L. Saturated FA decreased and unsaturated FA increased linearly with increasing dose of PTSO. The concentration of *trans*-10,*cis*-12 conjugated linoleic acid decreased by 78.5% with addition of PTSO at the highest dose (150 mg/L). Results suggest the potential of PTSO to modify ruminal fermentation in a direction consistent with higher propionate molar proportion, higher outflow of unsaturated FA, and low *trans*-10,*cis*-12 conjugated linoleic acid in an effective dose between 50 and 100 mg/L.

Key words: essential oil, fatty acid, garlic oil, rumen fermentation

INTRODUCTION

The ban on the use of antibiotics as growth promoters in animal feeds in the European Union (European Commission, 2003) has stimulated research on potential alternatives. Among them, essential oils seem promising because of their antimicrobial properties (Cowan, 1999). Garlic oil is a complex mix of many different compounds present in the plant or derived from processing and it has antimicrobial activity against a wide spectrum of bacteria (Calsamiglia et al., 2007). Several in vitro fermentation trials with rumen fluid reported that garlic oil reduced concentrations of acetate, branched-chain VFA (**BCVFA**), and ammonia-N, and increased concentrations of propionate and butyrate (Cardozo et al., 2004; Busquet et al., 2005a,b, 2006). Busquet et al. (2005b) investigated the effects of garlic oil and 4 of its main active compounds and found that diallyl disulfide and allyl mercaptan were the major compounds responsible for its action. However, chemical instability of these compounds may reduce the effectiveness of garlic oil in field conditions (Lawson and Gardner, 2005; Fujisawa et al., 2008).

Recently, 2 stable organosulfurate compounds of garlic have been purified (Lara Cambil and Garcia-Pareja, 2006): propyl-propane thiosulfinate (**PTS**) and propyl-

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propane thiosulfonate (**PTSO**). The compounds are structurally similar and differ only in the presence of one more oxygen function in PTSO. These compounds were first tested in vitro using goat rumen fluid and results suggested that they could be used to modify ruminal fermentation (Martínez-Fernández et al., 2013). Further, Ramos-Morales et al. (2013) tested the effects of PTS in continuous culture fermenters inoculated with goat rumen fluid and reported moderate increases in PUFA and *trans*-10 C18:1 concentrations in the 24-h effluent, suggesting the potential of garlic oil compounds to modify lipolysis and biohydrogenation in the rumen. However, PTSO demonstrated a stronger antimicrobial activity than PTS when tested in the gastrointestinal microbiota of pigs (Ruiz et al., 2010). The objective of the current study was to investigate the potential effects of PTSO on rumen microbial fermentation and FA profile in a dual-flow continuous culture system.

MATERIALS AND METHODS

Apparatus and Experimental Design

Eight 1,320-mL dual-flow continuous culture fermenters developed by Hoover et al. (1976) were used in 2 replicated periods. Each experimental period consisted of 5 d for adaptation of the rumen fluid to treatments and 3 d for sampling.

On the first day of each period, rumen fluid was taken from a dry Holstein cow (625 kg of BW) fed a 60% forage and 40% concentrate (DM basis) diet, using a probe connected to an automated vacuum pump. Rumen fluid from 5 different locations was collected into two 5-L thermoses under CO_2 flushing to maintain anaerobic conditions. Upon arrival at the laboratory, collected rumen fluid was filtered through 2 layers of cheesecloth to remove large feed particles, and then it was used, undiluted, to inoculate fermenters. Fermentation conditions were maintained constant with a temperature of 39°C, and pH at 6.4 ± 0.05 controlled by infusions of 3 *N* HCl or 5 *N* NaOH, and monitored by a computer and a Programmable Linear Controller (FieldPoint, National Instruments, Austin, TX). Anaerobic conditions were maintained by the infusion of N_2 gas at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks and contained 0.4 g/L of urea to simulate recycled N. Liquid and solid dilution rates were set at 0.10 and 0.05 h^{-1}, respectively.

Experiment 1. All fermenters were fed 95 g/d of DM of the diet (17% CP, 27% NDF, 15% ADF; 3.2% fat; DM basis) in 3 equal portions at 0700, 1500, and 2300 h. The diet was ground to pass a 1.5-mm screen (SM 2000, Retsch GmbH, Haan, Germany) and consisted (DM basis) of alfalfa hay (23.7%), corn silage (30.5%), ground corn grain (29.6%), soybean meal (15.4%), and a vitamin and mineral mixture (0.8%). The vitamin and mineral mixture contained per kilogram of DM: 300 g of MgO; 267 g of urea; 33 g of sulfur; 67 g of NaCl; 4,660 mg of Zn; 2,660 mg of Mn; 167 mg of Cu; 27 mg of Se; 33 mg of I; 7 mg of Co; 1,000 kIU of vitamin A; 200 kIU of vitamin D_3; and 1,330 mg of vitamin E.

Treatments included a negative control without additive (**CTR**), a positive control with monensin at 12 mg/L (**MON**; Sigma-Aldrich Chemical, St. Louis, MO) and 2 doses of PTSO (Pancosma SA, Geneva, Switzerland) at 30 mg/L (**PTSO30**) and 300 mg/L (**PTSO300**). Treatments were incorporated directly into the fermenter fluid 1 min before each feeding. Daily doses of PTSO30, PTSO300, and MON were dissolved in 1.2 mL of ethanol, and fermenters with the CTR treatment were supplied with 1.2 mL of ethanol in 3 doses daily.

Experiment 2. All fermenters were fed 95 g/d of DM of the diet (17% CP, 30% NDF, 19% ADF; 3.2% fat; DM basis) in 3 equal portions at 0700, 1500, and 2300 h. The diet was ground to pass a 1.5-mm screen (SM 2000, Retsch GmbH) and consisted (DM basis) of alfalfa hay (34.6%), corn silage (21.3%), ground corn grain (31.5%), soybean meal (12.0%), and the same vitamin and mineral mixture (0.8%) as in experiment 1. Treatments included a control without additive (**PTSO0**), and PTSO at 50 mg/L (**PTSO50**), 100 mg/L (**PTSO100**), and 150 mg/L (**PTSO150**). Treatments were incorporated directly into the fermenter fluid 1 min before each feeding. Daily doses of PTSO50, PTSO100, and PTSO150 were dissolved in 1.2 mL of ethanol, and fermenters with the PTSO0 treatment were also supplied with 1.2 mL of ethanol in 3 doses daily.

Sample Collection

During the last 3 d, 40 mL of filtered fermenter fluid was taken 2 h after the morning feeding to determine the concentrations of ammonia-N and VFA, trichloroacetic acid-soluble N (**TCA-N**), and tungstic acid-soluble N (**TA-N**). Results were used to calculate large peptides (**LPep** = TCA-N – TA-N), small peptides plus amino acids (**SPep** = TA-N – ammonia-N), and ammonia-N concentrations in fermenters (Winter et al., 1964).

During sampling days, effluent collection vessels were maintained at 4°C to prevent microbial activity. Solid and liquid effluents were mixed and homogenized for 1 min at 24,000 rpm (Diax900, Heidolph, Nurnberg, Germany), and a 500-mL sample was removed by aspiration and frozen at −20°C. Upon completion of each period, effluents from the 3 sampling days were composited