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The effects of cinnamaldehyde, monensin and quebracho condensed tannin on rumen fermentation, biohydrogenation and bacteria in continuous culture system

A. Ishlak[a], M. Günal[b], A.A. AbuGhazaleh[a,*]

[a] Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale, IL 62901, United States
[b] Department of Animal Science, Süleyman Demirel University, 32200 Isparta, Turkey

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ABSTRACT

The objective of this experiment was to evaluate the effects of different feed additives (cinnamaldehyde, monensin, and quebracho condensed tannin extract) on fermentation, *trans* fatty acids (FA) formation and selected strains of rumen bacteria. Four continuous culture systems were used in 4 × 4 Latin square designs with 4 periods of 10 days each. Treatment diets were: control diet (44:56 forage to concentrate; CON), control plus cinnamaldehyde (CIN) at 400 mg/L, control plus monensin (MON) at 12 mg/L, and control with quebracho condensed tannin extract (QTAN) at 100 g/kg of diet (DM basis). Fermenters were fed treatment diets three times daily at 120 g/day and overflow (effluent) samples were collected from each fermenter on days 8, 9 and 10 of each period to estimate nutrients digestibility and FA composition. On day 10 of each period, three samples were collected from each fermenter at 3 and 6 h post morning feeding for volatile fatty acids (VFA), ammonia-N and bacterial analyses. Compared with the CON diet, feed additives had no effects ($P > 0.05$) on apparent dry matter (DM), neutral detergent fiber (NDF) and organic matter (OM) digestibility but apparent protein digestibility decreased ($P < 0.01$) with the QTAN and CIN diets. Compared with the CON diet, the concentration of acetate decreased ($P < 0.05$) with the MON and CIN diets. The concentration of propionate increased ($P < 0.05$) with the MON and QTAN diets and was greatest with the MON diet. Ammonia-N concentration decreased ($P < 0.01$) with all feed additives and was least with the QTAN diet. The concentration of C18:0 decreased ($P < 0.01$) with the three feed additives and was least with the MON diet. Concentration of *trans* C18:1 and vaccenic acid (VA) increased ($P < 0.05$) with the MON and CIN diets and was greatest with the MON diet. Compared with the CON diet, the concentration of *c*9*t*11CLA increased ($P < 0.05$) only with the QTAN diet. The DNA abundance of *Butyrivibrio proteoclasticum* decreased ($P < 0.05$) with the MON and CIN diets while the DNA abundance for *Butyrivibrio* VA increased ($P < 0.05$) with all feed additives compared with the CON diet. Feed additives had no effects ($P > 0.05$) on the DNA abundance of *Anaerovibrio lipolytica* and *Butyrivibrio* SA. In conclusion, results demonstrate that the feed additives used in this study affected the fermentation and biohydrogenation process.

Abbreviations: ADF, acid detergent fiber; BH, biohydrogenation; CIN, cinnamaldehyde; CLA, conjugated linoleic acids; CON, control; CP, crude protein; DM, dry matter; EO, essential oils; FA, fatty acids; MON, monensin; NDF, neutral detergent fiber; OM, organic matter; QTAN, quebracho condensed tannin; VA, vaccenic acid; VFA, volatile fatty acids.

* Corresponding author at: Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale, Agriculture Bldg, Room 119, Carbondale, IL 62901-4417, United States. Tel.: +1 618 453 1767; fax: +1 618 453 5231.
E-mail address: aabugha@siu.edu (A.A. AbuGhazaleh).

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Addition of feed additives reduced the formation of C18:0 but only MON and CIN increased VA formation. MON and CIN effects on VA formation may in part be explained by their effects on *B. proteoclasticum*.

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1. Introduction

In recent years, a considerable amount of research work has focused on the fatty acid (FA) composition of ruminant products for the effects of their consumption on human health. Especially, FA such as conjugated linoleic acids (CLA) are active in the prevention of cancer, obesity and atherosclerosis in humans (Crumb, 2011). The most common CLA isomer is the *c*9*t*11 CLA, which is formed in the rumen during biohydrogenation (BH) of dietary C18:2*n*6 or in body tissue by Δ^9-desaturase from VA, another intermediate in ruminal BH of C18 unsaturated FA (Griinari et al., 2000).

Different dietary strategies have been used to decrease the rate of ruminal BH such as feeding plant and marine oils (AbuGhazaleh and Jacobson, 2007; Gudla et al., 2012), or algae (AbuGhazaleh et al., 2009) and increasing forage to concentrate ratio (AbuGhazaleh and Jacobson, 2007). Several feed additives have been also proposed to increase the content of CLA in milk fat. Ionophores such as monensin are known to reduce methane production by inhibiting the growth of gram-positive bacteria that produce hydrogen and that may interfere with the process of ruminal BH. Ionophores have been shown to decrease the rate of ruminal BH of unsaturated FA *in vitro* (Fellner et al., 1997) and increase the content of CLA in milk fat (AlZahal et al., 2008). However, the use of ionophores as a feed antibiotic in livestock has been banned in certain counties (*e.g.* EU) and criticized by others because of the possible presence of residues in food and the emergence of resistant strains of bacteria. Therefore, plant secondary metabolites such as essential oils (EO), saponins and tannins have been suggested as a potential means to manipulate bacterial populations involved in ruminal BH to modify the FA composition of ruminant-derived food products such as milk and meat. Although several studies have examined plant secondary metabolites effects on rumen fermentation and the greenhouse gases production (Makkar et al., 1995; Sliwinski et al., 2002; Cardozo et al., 2004; Busquet et al., 2006; Chaves et al., 2008; Hassanat and Benchaar, 2012), only few studies evaluated the effects of these compounds on BH. Additionally, little information is currently available about the effects of these plant metabolites on rumen microbial ecology, particularly, bacterial species believed to be involved in the BH process. Therefore, the main objective of this study was to evaluate the effects of two secondary metabolites such as QTAN from quebracho trees (*Schinopsis balansae*) and cinnamaldehyde and monensin on rumen *trans* FA formation and selected strains of rumen bacteria using continuous culture systems.

2. Materials and methods

2.1. Experimental design

Four 1700 ± 12 mL continuous culture fermenters (Stern and Hoover, 1990) were used in 4 × 4 Latin square designs with 4 periods of 10 days each. The first 7 days were used for adaptation and last 3 days for samples collection. Treatment diets were fed at 120 g/day (DM basis) in three equal portions during the day at 0800, 1500 and 2200 h. The diets were: (1) 44:56 forage to concentrate (CON), (2) CON plus cinnamaldehyde at 400 mg/L (1200 mg/day) (CIN), (3) CON plus monensin at 12 mg/L (36 mg/day) (MON), and (4) CON with quebracho condensed tannin at 100 g/kg of diet DM (QTAN; Tables 1 and 2). The forage consisted of grass hay while the concentrate mix contained corn, soybean meal, soy hulls, corn oil and minerals. Quebracho condensed tannin (purity of 0.70; Tannin Corporation, Peabody, MA, USA) was added to the diet by partially replacing some of the hay, corn and soy hulls. Cinnamaldehyde (C_9H_8O, purity of 0.98, Sigma–Aldrich, St. Louis, MO, USA); and monensin (Acros Organics Company, NJ, USA) were dissolved in ethanol and added directly into each fermenter with each feeding.

2.2. Continuous culture

Ruminal fluid was collected from a fistulated lactating Holstein cow fed (55:45 forage to concentrate diet; DM basis). At 2–4 h after the morning feeding, ruminal contents were collected into a plastic bag under anaerobic conditions. The rumen contents brought to the laboratory; were strained through 2 layers of cheesecloth, and used within 15 min. Approximately 1300 mL of the ruminal fluid were added to each of the four fermenters, containing 400 mL of prewarmed buffer. Cultures were stirred continuously at 120 rpm *via* a magnetic impeller stirrer unit and fermenter pH was maintained above 6.2 by adjusting buffer pH level with 1 N NaOH or 1 N HCl. Fermenters pH was measured daily before feeding using a portable pH meter at 0800, 1500 and 2400. Buffer was delivered continuously at a flow rate of 1.16 mL/min (0.10 h^{-1} liquid dilution rate), using a precision pump. Anaerobic conditions in fermenters were maintained by purged with N_2 gas (80 mL/min) and fermenter temperature was maintained at 39 °C. Flow rate of each fermenter was recorded every day at 08:00.

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