



Effects of ruminal doses of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells

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ABSTRACT

The objective was to evaluate effects of a ruminal dose of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells. Six ruminally cannulated nonlactating nonpregnant Holstein cows (body weight = 725 ± 69.6 kg) were assigned to treatments in a 3 × 3 Latin square design with 7-d periods; 1 d for data and sample collection followed by a 6-d washout period. Cows were fed a diet containing whole-crop barley silage and dry ground corn, and dietary neutral detergent fiber and crude protein contents were 41.8 and 13.2% [dry matter (DM) basis], respectively. Treatment was a pulse-dose of sucrose, lactose, and corn starch (3.0, 3.0, and 2.85 kg of DM, respectively; providing similar amounts of hexose across the treatments) through the ruminal cannulas. All treatments were given with alfalfa silage (1.75 kg DM) to prevent acute rumen acidosis. Rumen pH was continuously monitored, and rumen fluid was sampled at 0, 30, 60, 90, 120, 150, and 180 min after the dose. In addition, ruminal papillae were sampled from the ventral sac at 180 min after the dose. Ruminal dosing with sucrose and lactose, compared with corn starch, increased ruminal total volatile fatty acid concentration and molar proportion of butyrate from 60 to 180 min after the dose, and expression of genes for sodium hydrogen exchanger isoforms 1 and 2, and ATPase isoform 1 in ruminal epithelial cells. Ruminal dosing with sucrose, compared with lactose and corn starch, decreased rumen pH from 120 to 180 min after the dose and molar proportion of acetate in ruminal fluid from 60 to 150 min after the dose, and increased molar proportion of propionate in ruminal fluid from 60 to 150 min, and expression of genes involved in butyrate metabolism (3-hydroxy-3-methylglutaryl-coenzyme A synthase isoform 1) and anion exchange across ruminal apical cell membrane (putative anion transporter isoform 1). These results suggest that replacing dietary starch with sugars may affect ruminal fermentation and

metabolism regulating intracellular pH and fermentation acid absorption in ruminal epithelial cells, and that these effects can be greater for sucrose than lactose.

Key words: sucrose, lactose, starch, volatile fatty acids, gene expression

INTRODUCTION

Sugars are part of the carbohydrates fed in diets of lactating dairy cows. Sugars ferment quickly in the rumen; Sniffen et al. (1992) estimated that sugars ferment at 300%/h, whereas the fermentation rate of starch varies from 15 to 40%/h depending on grain types and processing methods. Weisbjerg et al. (1998) showed that hydrolysis rates of sugars varied from 248 to 1,404%/h, and that they ferment almost completely (i.e., >95%) in the rumen. Replacing dietary starch with sugars often increases DMI (DeFrain et al., 2004; Broderick et al., 2008; Penner and Oba, 2009) and milk fat production (Broderick et al., 2008; Penner and Oba, 2009); however, its mode of action is not well understood. Despite rapid fermentation, according to a review by Oba (2011), the majority of in vivo studies reported that rumen pH is not affected by feeding sugars, and a few studies reported that replacing a dietary starch source with sugars increased rumen pH (Chamberlain et al., 1993; Heldt et al., 1999) or tended to increase rumen pH (Penner et al., 2009; Penner and Oba, 2009). In addition, the effects of feeding sugars on the VFA profile in rumen fluid are not consistent. Although in vitro studies suggested that sugar fermentation increases butyrate production (Vallimont et al., 2004; Ribeiro et al., 2005), in vivo studies reported considerably variable effects of sugar feeding on VFA profile as summarized by Oba (2011).

The discrepancy between expected and observed rumen fermentation variables can be partly attributed to data and sample collection protocols. Rumen pH data are usually measured continuously and summarized on a daily basis to account for diurnal variation. Similarly, when the VFA profile of rumen fluid is determined, in most studies, multiple samples are composited to account for diurnal variation. However, this data may not reflect how sugars ferment in the rumen because

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most of dietary sugars are expected to ferment immediately after consumption by animals. In addition, the absorption rate of butyrate is faster than that of acetate or propionate (Leek, 1993), and it may be difficult to detect increases in butyrate concentration in rumen fluid even if sugar fermentation increases butyrate production in the rumen. Furthermore, butyrate concentration in rumen fluid may not necessarily reflect its production because the former is also affected by absorption and passage of butyrate.

It is necessary to identify short-term effects of feeding sugars on rumen fermentation to elucidate mechanisms on how sugar feeding affects animal performance. In addition, although ruminal epithelial metabolism can affect VFA absorption and rumen pH (Aschenbach et al., 2011), effects of feeding sugars on the metabolism of ruminal epithelial cells have not been extensively studied. Furthermore, effects of sugar type on ruminal fermentation and metabolism in ruminal epithelial cells are not well understood. As such, objectives of the current study were to determine short-term effects of ruminal doses of sucrose and lactose on rumen fermentation and gene expression in ruminal epithelial cells compared with ruminal doses of starch, the most common NFC in dairy diets.

MATERIALS AND METHODS

The current study was conducted from June 2013 to July 2013 at the University of Alberta Dairy and Research Technology Center. Animals used in this study were cared for in agreement with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada). All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock (# AUP580).

Experimental Design

Six multiparous nonlactating nonpregnant Holstein cows (BW 725 ± 69.6 kg; mean \pm SD) were used in this study. All cows were ruminally cannulated for previous studies, and were housed in individual stalls bedded with wood shavings. The cows were fed a TMR (Table 1), once daily at 0800 h, ad libitum allowing for 5% refusals throughout the trial, and had free access to water. Dry matter intake was 12.2 ± 0.79 kg/d (mean \pm SD) during the study. After 21-d diet adaptation, cows were assigned to treatments in a duplicated 3×3 Latin square design, balanced for carryover effects, with 7-d periods; 1 d for data and sample collection, followed by a 6-d washout period. Treatments were ruminal doses of corn starch, sucrose, and lactose (2.85, 3.00, and 3.00 kg of DM, respectively, to provide the

Table 1. Ingredients and chemical composition of the diet fed during the study

Item	Measurement
Ingredients composition, % of DM	
Barley silage ¹	82.6
Dry ground corn	5.6
Canola meal	5.3
Sucrose	2.1
Lactose	2.1
Salt	0.8
Canola oil	0.6
Limestone	0.4
Calcium diphosphate	0.4
Magnesium oxide	0.2
Vitamin ADE premix ²	0.019
Selenium ³	0.008
Trace mineral premix ⁴	0.007
Nutrient composition	
DM, %	39.3
CP, % of DM	11.4
NDF, % of DM	40.4
NFC, % of DM	38.1

¹DM: 34.9%; CP: 14.4%DM; NDF: 51.8%DM; starch 9.8%DM.

²Contained 60,000 kIU/kg of vitamin A, 6,000 kIU/kg of vitamin D, 20 kIU/kg of vitamin E.

³Contained 2,000 mg/kg of Se.

⁴Contained 133,400 mg/kg of Cu, 240,000 mg/kg of Mn, 2,700 mg/kg of Co, 20,000 mg/kg of Zn, and 6,000 mg/kg of I.

same amount of hexoses). On each data collection day, treatment carbohydrates were manually dosed into the rumen at 0800 h with 1.75 kg of alfalfa silage (DM basis), to minimize the risk of rumen acidosis. Data and samples were collected for 3 h following the ruminal dose, and cows were subsequently fed the diet at 1130 h after data and sample collection was completed.

Data and Sample Collection

Rumen pH was measured every 30 s over a 4-h period, from 1 h before the treatment dose to 3 h after the dose, using the Lethbridge Research Center ruminal pH measurement system (Penner et al., 2006), and summarized for each hour. Rumen fluid was sampled, every 30 min (0, 30, 60, 90, 120, 150, and 180 min relative to the ruminal dose of treatment), from 5 locations in the rumen (cranial dorsal, cranial ventral, central, caudal dorsal, and caudal ventral) and strained through a perforated fabric screen (WeedBlock, Easy Gardener, Waco, TX). Samples were placed on ice immediately, and centrifuged at $3,000 \times g$ for 20 min at 4°C. The supernatant was stored at -20°C until analysis, and subsequently analyzed for VFA profile as described by Khorasani et al. (1996).

Immediately after the last rumen fluid collection (180 min after the treatment dose), rumen contents were evacuated into an insulated container. Then, the ventral sac region of the rumen was pulled out through the rumen cannula, and approximately 30 papillae

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