J. Dairy Sci. 97:1–20
http://dx.doi.org/10.3168/jds.2014-8003

Ruminal bacterial community shifts in grain-, sugar-, and histidine-challenged dairy heifers

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ABSTRACT

Ruminal bacterial community composition (BCC) and its associations with ruminal fermentation measures were studied in dairy heifers challenged with combinations of grain, fructose, and histidine in a partial factorial study. Holstein-Friesian heifers (n = 30) were randomly allocated to 5 triticale grain-based treatment groups: (1) control (no grain), (2) grain [fed at a dry matter intake (DMI) of 1.2% of body weight (BW)], (3) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI), (4) grain (1.2% of BW DMI) + histidine (6 g/head), and (5) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head). Ruminal fluid was collected using a stomach tube 5, 115, and 215 min after consumption of the rations and bacterial 16S ribosomal DNA sequence data was analyzed to characterize bacteria. Large variation among heifers and distinct BCC were evident in a between-group constrained principal components analysis. Bacterial composition in the fructose-fed heifers was positively related to total lactate and butyrate concentrations. Bacterial composition was positively associated with ruminal ammonia, valerate, and histamine concentrations in the grain-fed heifers. The predominant phyla were the *Firmicutes* (57.6% of total recovered sequences), *Bacteroidetes* (32.0%), and candidate phylum TM7 (4.0%). *Prevotella* was the dominant genus. In general, grain or histidine or their interactions with time had minimal effects on the relative abundance of bacterial phyla and families. Fructose increased and decreased the relative abundance of the *Firmicutes* and *Proteobacteria* phyla over time, respectively, and decreased the abundance of the *Prevotellaceae* family over time. The relative abundance of the *Streptococcaceae* and *Veillonellaceae* families was increased in the fructose-fed heifers and these heifers over time. A total of 31 operational taxonomic units differed among treatment groups in the 3.6 h sampling period, *Streptococcus bovis* was observed in fructose fed animals. The TM7 candidate phylum had an increased abundance of sequence reads by over 2.5 fold due to the introduction of histidine into the diet. Rapid changes in BCC can occur in a short period after a single substrate challenge and the nature of these changes may influence ruminal acidosis risk and differ from those in cattle exposed to substrate challenges over a longer time period.
Key words: bacterial community composition, fructose, histidine, ruminal acidosis

INTRODUCTION

The rumen ecosystem is highly responsive to dietary changes, most notably those that occur during weaning and shifts from forage- to concentrate-based diets (Tajima et al., 2000). Examples of marked dietary change occur at the transition period in dairy systems or induction of beef cattle to feedlot diets. These abrupt changes in diet can be associated with ruminal acidosis, a complex of ruminal conditions with a significant economic impact (Stone, 1999). The complex occurs along a continuum of severity, reflected in increased disease and losses in production performance. The prevalence of ruminal acidosis has been reported as approximately 20% in Wisconsin dairy herds (Oetzel et al., 1999), 10% in Australian herds (Bramley et al., 2008), and 11% in Irish herds (O'Grady et al., 2008). Bramley et al. (2008) found diets with high NFC and low NDF increased the risk of ruminal acidosis, a condition associated with increased ruminal propionate, valerate, butyrate, and acetate and decreased ammonia concentrations and lower milk fat-to-protein ratio.

Feeding different substrates alters ruminal fermentation responses (Heldt et al., 1999; Golder et al., 2012), presumably partly resulting from different responses in bacterial community composition (**BCC**) in the rumen. Researchers have examined ruminal bacterial composition changes among cattle fed forage- and concentrate-based diets (Tajima et al., 2000; Petri et al., 2012), cattle fed increasing dietary percentages of concentrate (Callaway et al., 2010; Fernando et al., 2010), or in

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Received January 31, 2014.
Accepted April 10, 2014.
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cattle with induced subacute acidosis (Khafipour et al., 2009; Hook et al., 2011). However, these studies have used relatively small numbers of cattle and focused on changes that occurred after adaptation to feed changes. Others have suggested that the rumen microbiome is reasonably resistant to dietary changes (Weimer et al., 2010) and a change in the microbiome is not always related to the severity of ruminal acidosis (Mohammed et al., 2012).

Golder et al. (2012) hypothesized that the addition of 0.4% of BW fructose or histidine (6 g per head), or both, to grain fed as a single challenge would increase the onset of SARA. The rationale for this hypothesis is that fructose is rapidly metabolized compared with starch (Firkins, 2011) and metabolic acidosis was induced in heifers drenched with 13, 17, or 21 g/kg (~0.13, 0.17, and 0.21% of BW) of oligofructose, a polymer of fructose (Thoefner et al., 2004). Ruminal and systemic acidosis were also induced when 17 g/kg (~0.17% of BW) of oligofructose was administered to heifers (Danscher et al., 2009; 2010). With increased interest in potential benefits of *Lolium perenne* varieties with greater water-soluble carbohydrate content in pasture-based dairying (Miller et al., 2001; Tas et al., 2006), it is important to assess the role of sugars in the development of acidosis. The concentration of fructose fed by Golder et al. (2012; 0.4% of BW) is similar to concentrations of water-soluble carbohydrates ingested by cattle over a day.

Release of histamine has been hypothesized to have an important role in acidosis (Dain et al., 1955; Ahrens, 1967), as has endotoxin release (Gozho et al., 2005; Khafipour et al., 2009). The amino acid histidine is decarboxylated at low rumen pH by the bacteria *Allisonella histaminiformans* to produce the inflammatory molecule histamine (Garner et al., 2002). The rationale for use of histidine in the study was based on the relatively high concentrations in white clovers, ryegrass, and kikuyu (Reeves et al., 1996; Penkov et al., 2003). Providing fructose and histidine in combination may have a different influence on BCC and subsequent rumen fermentation measures compared with when they are fed in isolation.

Golder et al. (2012) found that a grain challenge decreased ruminal pH and increased ammonia, total VFA, acetate, butyrate, propionate, and valerate concentrations compared with the controls fed no grain. Fructose fed at 0.4% of BW decreased ruminal pH and valerate concentrations, increased total VFA and butyrate concentrations, and markedly increased D- and L-lactate concentrations compared with non-fructose-fed heifers. Histidine had limited effects on ruminal fermentation. Combinations of grain, fructose, and histidine had no effects on ruminal endotoxin and plasma oxidative stress biomarker concentrations (Golder et al., 2013). It was concluded that the fructose-fed heifers were at the highest risk of SARA.

The aim of this study was to examine the effects of combinations of grain, fructose, and histidine, fed to dairy heifers unadapted to these in a single challenge, on ruminal BCC and its associations with ruminal fermentation measures. We hypothesized that distinct bacterial communities would begin to develop over 3.6 h after the single challenge feeding among heifers fed combinations of grain, fructose, and histidine and reflect ruminal fermentation measures. We wished to evaluate responses in the context of the existing understanding of ruminal acidosis models commonly presented (Owens et al., 1998; Nagaraja and Titgemeyer, 2007; RAGFAR, 2007).

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted on 30 nonpregnant Holstein heifers <18 mo of age (359.3 ± 47.3 kg of BW) at Camden, New South Wales (**NSW**), Australia. All experimental procedures were approved by the Bovine Research Australasia Animal Ethics Committee (BRA 0609-0610). The experiment consisted of a 10-d adaptation period followed by a single-pulse feeding challenge on d 11. All heifers were housed on a dry lot and fed twice daily on a feed pad with individual head stanchions. During the adaptation period, the heifers were fed 1 kg (as fed) of grain daily and a target intake of 7.2 kg of alfalfa hay/d and 2 kg of ryegrass silage/d (as-fed basis). Rumen and blood samples were collected over approximately a 3.6-h period after consumption of the challenge rations. Dietary information, experimental detail, and ruminal fermentation products, ruminal pH, endotoxin, and oxidative stress results have been reported previously (Golder et al., 2012, 2013). This article provides data on BCC and interprets other findings in this context.

Treatment Groups

As described by Golder et al. (2012), 30 Holstein-Friesian heifers (n = 6 heifers/group) were randomly assigned to 5 treatment groups in a partial factorial arrangement: (1) control (no grain), (2) grain (fed at a crushed grain DMI of 1.2% of BW), (3) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI), (4) grain (1.2% of BW DMI) + histidine (6 g/head), and (5) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head). The chemical composition of the grain, triticale cultivar Berkshire, was analyzed by wet