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A single mild episode of subacute ruminal acidosis does not affect ruminal barrier function in the short term

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ABSTRACT

Twenty-four German Merino sheep (72.3 ± 10.1 kg of body weight) were fed an all-hay diet and assigned to either the subacute ruminal acidosis (SARA) treatment (n = 17) or sham treatment (n = 7). The SARA sheep were orally dosed with a 2.2 *M* glucose solution to supply 5 g of glucose/kg of body weight, whereas sham sheep received an equal volume of water. Ruminal pH was measured for 48 h before and 3 h after the oral dose. Sheep were then killed and ruminal epithelia from the ventral sac were mounted in Ussing chambers. The serosal-to-mucosal flux rate of partially ^{3}H-labeled mannitol ($J_{\text{mannitol-SM}}$), an indicator of barrier function, was measured while epithelia were exposed to 3 sequential in vitro measurement periods lasting 1 h each. The measurement periods consisted of baseline, challenge, and recovery periods and were interspersed by 30-min periods for treatment equilibration. Baseline conditions were pH 6.1 (mucosal solution) and pH 7.4 (serosal solution) with a bilateral osmolarity of 293 mOsm/L. During the challenge period, the mucosal side of the epithelia was exposed to either an acidotic challenge (pH 5.2, osmolarity 293 mOsm/L) or an osmotic challenge (pH 6.1, osmolarity 450 mOsm/L); a third group served as control (pH 6.1, osmolarity 293 mOsm/L). The mucosal buffer solution was replaced for the recovery period. In vivo, sheep on the SARA treatment had lower mean (5.77 vs. 6.67) and nadir (5.48 vs. 6.47) ruminal pH for the 3 h following the oral drench compared with sham sheep, indicating the successful induction of SARA with the oral glucose dose. Despite the marked reduction in pH in vivo, induction of SARA had no detectable effects on the baseline measurements of $J_{\text{mannitol-SM}}$, tissue conductance (G_t), and short-circuit current (I_{sc}) in vitro. However, reducing mucosal pH to 5.2 in vitro had negative effects on epithelial barrier function in the recovery period, including increased $J_{\text{mannitol-SM}}$, increased G_t, and decreased I_{sc}. The osmotic challenge increased $J_{\text{mannitol-SM}}$ and G_t and decreased I_{sc} during the challenge period, which was reversible in the recovery period except for slight reduction in I_{sc}. Interactions between the in vitro treatment and measurement period were detected for $J_{\text{mannitol-SM}}$, G_t, and I_{sc}. These data indicate that a mild episode of SARA (nadir pH, 5.48; duration ruminal pH <5.8, 111 min relative to the 180-min measurement period) does not affect ruminal epithelial barrier function immediately after the episode but that a rapid and more severe acidification (pH 5.2) in vitro increases epithelial permeability following the insult.
Key words: barrier function, ruminal acidosis, ruminal epithelium, Ussing chamber

INTRODUCTION

The accumulation and dissociation of short-chain fatty acids (**SCFA**) in ruminal fluid decreases pH and can lead to the onset of ruminal acidosis (Owens et al., 1998; Plaizier et al., 2008). Because of the current energy-intensive feeding regimens, ruminal acidosis is a persisting disorder in dairy and beef cattle. Ruminal acidosis has negative consequences on feed efficiency through decreased fiber digestibility and impaired production efficiency (Stone, 2004). In addition, ruminal acidosis has been linked to both morphological and histological alterations in ruminal papillae (Steele et al., 2009). The prominent histological alterations during acute and repeated episodes of ruminal acidosis strongly suggest an impaired barrier function (Steele et al., 2009) that may provide the explanation for the translocation of toxins and bacteria during the disorder (Plaizier et al., 2008).

Past studies examining the effect of pH on ruminal epithelial function have consistently demonstrated that epithelial exposure to pH values commonly used for the diagnosis of acute ruminal acidosis (pH ≤5.1) results in the rapid reduction of epithelial barrier function

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(Gaebel et al., 1987; Gaebel et al., 1989; Aschenbach and Gäbel, 2000). In studies with a chronic exposure to low ruminal pH, both morphological and histological alterations in ruminal papillae were evident, suggesting reduced barrier function (Steele et al., 2009). Although there is fundamental knowledge of the consequences of acute ruminal acidosis on ruminal epithelial function (Ahrens, 1967; Gaebel et al., 1987; Gaebel and Martens, 1988), only a limited number of studies have examined the functional consequences of a transient and mild acidotic challenge (i.e., SARA) on the ruminal epithelia. However, it is SARA that constitutes the most prevalent form of ruminal acidosis in current dairy production systems (Krause and Oetzel, 2006).

Past studies examining ruminal epithelia function after exposure to low pH have largely focused on absorptive functions. For example, Gaebel and Martens (1988) showed that under washed reticulorumen conditions, an exposure of epithelia to a luminal pH of 5.4 only transiently decreased Na^+ net absorption and transmural potential difference. In an in vitro study, Gaebel et al. (1989) demonstrated that mucosal exposure to buffer with a pH value of 6.0 had no effect on short-circuit current ($\boldsymbol{I_{sc}}$) or tissue conductance ($\boldsymbol{G_t}$) but further reduction to pH 5.5 decreased I_{sc} and increased G_t, suggesting reduced ion transport and increased epithelial permeability, respectively. With respect to barrier function, Emmanuel et al. (2007) demonstrated that a mucosal pH of 5.5 in vitro had no effect on mannitol or lipopolysaccharide translocation across the ruminal epithelium, whereas Aschenbach and Gäbel (2000) showed that a mucosal exposure to pH 5.4 increased the mucosal-to-serosal flux of histamine across ruminal epithelia in vitro.

Because ruminal acidosis entails more than simply reducing ruminal pH [e.g., increased osmolarity (Carter and Grovum, 1990; Owens et al., 1998) and increased SCFA and toxin concentrations (Plaizier et al., 2008)], it should be acknowledged that any one or the combination of these factors may affect epithelial barrier function. Past studies have investigated the specific effect of low pH (typical of acute ruminal acidosis), hyperosmolarity (Gaebel et al., 1987; Gaebel and Martens, 1988; Schweigel et al., 2005; Lodemann and Martens, 2006), or an exposure to toxins (Aschenbach and Gäbel, 2000; Emmanuel et al., 2007) in vitro. Because SARA rather than acute ruminal acidosis is common in dairy cattle (Krause and Oetzel, 2006), the objective of this study was to elucidate whether exposure of the ruminal epithelium to a short episode of SARA in vivo has persistent effects on the barrier function. We further aimed to determine whether such persistent effects could include altered responses to subsequent episodes of mucosal acidity and hyperosmolarity in vitro. We hypothesized that inducing SARA in vivo would compromise ruminal epithelial barrier function, with subsequent in vitro challenges leading to a further reduction in barrier function.

MATERIALS AND METHODS

This is one paper in a series arising from a single experiment that aimed to evaluate the susceptibility of individual animals to ruminal acidosis. As such, detailed experimental procedures have been described previously (Penner et al., 2009b). This study was conducted between April and August 2008 at the Universität Leipzig (Leipzig, Germany). All procedures were preapproved by the Regierungspräsidium Leipzig (TVV 06/08) and the Faculty Animal Policy and Welfare Committee at the University of Alberta (Edmonton, Alberta, Canada) and were in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Animals and Experimental Design

Twenty-four German Merino sheep (72.3 ± 2.6 kg of BW; mean ± SD) were used as a model for ruminants. Sheep were sourced from 2 locations and were fed an all-hay diet ad libitum for at least 21 d before the start of the experiment. On a DM basis, the hay contained 13.1% CP and 8.1 MJ/kg of ME. Sheep had free access to water and a salt and mineral block.

Prior to the experiment, sheep were transferred to a pen bedded with wood shavings. Hay, water, and mineral block were withdrawn at 0600 h and sheep were randomly exposed to either the control treatment (referred to as sham; n = 7) or the SARA challenge treatment (referred to as SARA; n = 17). Sheep were weighed and SARA was induced using a ruminal infusion of a 2.2 *M* glucose solution to supply 5 g of glucose/kg of BW. The infusion was administered using an orogastric tube (12 mm o.d., 150 cm long; Heiland Vet GmbH, Hamburg, Germany). Sheep receiving the sham treatment were exposed to the same procedure but received an equivalent volume of water instead of glucose solution.

Continuous Ruminal pH Measurement in Vivo

The protocol for the measurement of ruminal pH in these sheep has previously been reported (Penner et al., 2009b). Briefly, an orally dosable small ruminant ruminal pH measurement system (Penner et al., 2009a; Dascor, Escondido, CA) was used to measure ruminal pH starting 48 h before the oral drench extending for 3 h following the oral drench (Penner et al., 2009b).