Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis

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1Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada; 2Department of Poultry Science, University of North Carolina, Raleigh, North Carolina; and 3Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada

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Steele MA, Croom J, Kahler M, AlZahal O, Hook SE, Plaizier K, McBride BW. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. Am J Physiol Regul Integr Comp Physiol 300: R1515–R1523, 2011. First published March 30, 2011; doi:10.1152/ajpregu.00120.2010.—Alterations in rumen epithelial structure and function during grain-induced subacute ruminal acidosis (SARA) are largely undescribed. In this study, four mature nonlactating dairy cattle were transitioned from a high-forage diet (HF: 0% grain) to a high-grain diet (HG: 65% grain). After feeding the HG diet for 3 wk, the cattle were transitioned back to the original HF diet, which was fed for an additional 3 wk. Continuous ruminal pH was measured on a weekly basis, and rumen papillae were biopsied during the baseline and at the first and final week of each diet. The mean, minimum, and maximum daily ruminal pH were depressed (P < 0.01) in the HG period compared with the HF period. During the HG period, SARA was diagnosed only during week 1, indicating ruminal adaptation to the HG diet. Microscopic examination of the papillae revealed a reduction (P < 0.01) in the stratum basale, spinosum, and granulosum layers, as well as total depth of the epithelium during the HG period. The highest (P < 0.05) papillae lesion scores were noted during week 1 when SARA occurred. Biopsied papillae exhibited a decline in cellular junctions, extensive sloughing of the stratum corneum, and the appearance of undifferentiated cells near the stratum corneum. Differential mRNA expression of candidate genes, including desmoglein 1 and IGF binding proteins 3, 5, and 6, was detected between diets using qRT-PCR. These results suggest that the structural integrity of the rumen epithelium is compromised during grain feeding and is associated with the differential expression of genes involved in epithelial growth and structure.

epithelium; structure; stratified squamous epithelium; structure; gene expression

It has become common to feed diets rich in rapidly fermentable nonstructural carbohydrates (high-grain) to increase feed conversion in intensive ruminant agriculture systems (46, 49). When ruminants are fed excessive levels of grain, rumen fermentation of carbohydrates can exceed the rate of ruminal absorption and buffering, causing an accumulation of short-chain fatty acids (SCFA). When ruminal pH is depressed below 5.6 for more than 3 h/day, the disease state of subacute ruminal acidosis (SARA) begins to develop (1, 33, 46). SARA is associated with major changes in the populations of rumen microflora (30), ruminal SCFA fermentation patterns, altered gastrointestinal function, feed intake, milk production and composition, and liver abscesses (53). The negative economic consequences of poor performance and animal health caused by SARA have made it one of the most prevalent animal welfare issues in intensive ruminant production systems (64).

The ruminant forestomach is essential for the fermentation of structural carbohydrates and plays a critical role in the absorption of SCFA across the stratified squamous rumen epithelium (SSE) (15). Papillae protruding from the rumen wall greatly increase the surface area for absorption of SCFA, which accounts for 75% of the metabolizable energy supply (8). The ruminal SSE is composed of four distinct strata with multiple functions (21, 63). Adjacent to the basal lamina are the stratum basale and spinosum, which have functional mitochondria contributing to the metabolic properties of the ruminal SSE, primarily the production of ketones from SCFA (35). The stratum granulosum is adjacent to the stratum spinosum and is characterized by junctional complexes termed desmosomes acting as the permeability barrier of the SSE (5, 16, 21). Desmosomes are composed of multiple proteins (desmosglein, desmocollin, desmoplakin, plakoglobin, and plakophilin), which link intermediate filaments to sites of intercellular adhesions ultimately to add mechanical strength to the epithelium (22, 25). The stratum corneum is in direct contact with the rumen milieu and consists of cornified keratinocytes commonly referred to as horn cells (34). It acts as a physical protective barrier between the rumen milieu and the lower viable strata (63).

The rumen has been shown to adapt to increasing levels of dietary grain by increasing the size of the papillae, thus maximizing the surface area for SCFA absorption (17, 18, 47). In sheep and cattle, increasing dietary grain or SCFA concentrations can rapidly increase ruminal SSE proliferation (18, 59) and morphogenesis (62). Of the SCFAs, butyrate is thought to stimulate cellular proliferation events (54) and inhibit apoptosis (41). In cattle fed high-grain diets, it has been suggested that the rate of cellular aging decreases and the SSE can become parakeratotic (65). Parakeratosis and hyperkeratosis are commonly associated with high-grain feeding and severely compromise SCFA absorption (11, 24). Ruminal acidosis can also cause erosion of the SSE, enabling translocation of rumen microbes into the portal bloodstream, thus causing liver abscesses (44). It has recently been shown that grain-induced SARA is associated with increased translocation of microbial LPS from the rumen and intestine into the blood, causing levels of proinflammatory cytokines and acute phase proteins to become elevated in dairy cattle (13, 20, 31, 32). Despite these documented consequences of compromised SSE function, it remains unknown how SSE structure and function adapt during SARA.
Little research has been conducted to uncover the molecular mechanisms governing ruminal SSE adaptation to high-grain diets. Candidate growth factors that have been shown to increase rumen epithelial cell proliferation in vitro include IGF and EGF (4). Both IGF and EGF induce their cellular response by binding to their respective cellular membrane receptors, thereby initiating a signal transduction cascade that turns on genes responsible for proliferation (27). Rumen papillae proliferation in goats fed high-energy diets has been associated with increased plasma IGF-I and IGF-I receptor expression in rumen tissue (59). However, IGF signaling is modulated by six known IGF binding proteins (IGFBP), which play a critical role in epithelial proliferation and differentiation in many intestinal epithelial cell models (14). Testing the expression of these candidate genes is a first step in elucidating the molecular based mechanisms of ruminal SSE adaptation to high-grain diets.

To develop a full understanding of this syndrome, it is necessary to characterize how ruminal acidosis and increased SCFA affect SSE histology and ultrastructure. The primary objective of this study was to characterize the microarchitectural adaptations of the rumen epithelium during SARA using light and electron microscopy imaging techniques for histological and ultrastructural analysis. The secondary objective was to characterize the expression of candidate genes and to begin identifying molecular markers with structural and morphoregulatory properties that may be related to functional changes in ruminal SSE during SARA. We hypothesized that the structural integrity of the rumen epithelium would be compromised during SARA and associated with differential expression of genes associated with growth (IGF and EGF gene families) and structure (desmosome gene family).

MATERIALS AND METHODS

Management of animals and experimental design. The outline of the experiment design and treatments has been described by Steele et al. (61). Briefly, four mature (9.3 ± 2.2 yr, age ± SD) rumen-cannulated, nonpregnant, and nonlactating Holstein dairy cows [760 ± 30 kg, body weight (BW) ± SD] were used in this experiment, conducted in the dairy barn at the University of Guelph (Guelph, Ontario, Canada). All experimental procedures were conducted within the guidelines of the Canadian Council on Animal Care and were approved by the University of Guelph Animal Care Committee. Prior to the experiment, the cattle were fitted with rumen cannulas (12), acclimatized to the experimental environment, and consumed a high-forage (HF) diet consisting exclusively of chopped hay (90.6% dry matter, 114 g crude protein/kg dry matter, 600 g neutral detergent fiber/kg dry matter, 173 g nonfiber carbohydrate/kg dry matter, 69 g starch/kg dry matter; 1.91 mCal/kg dry matter) for 45 days to acclimatize to the experimental environment before initiating a baseline week of ruminal fluid pH measurements (45) for nonlactating cattle. Since the ventral sac has the highest capillary blood flow per unit weight mucosa of any location within the rumen, it was chosen as the biopsy site (66). Approximately 150 mg of rumen papillae were collected from exclusive sites in the ventral sac during each biopsy and washed in ice-cold PBS (pH = 7.4, 1X) 20 times prior to immediate fixation or freezing.

Microscopy. Five papillae were prepared for light microscopy histomorphometric analysis using methods previously described by Odongo et al. (47). Formalin-fixed, paraffin-embedded papillae were sectioned at a 4-µm thickness, stained with hematoxylin and eosin, and mounted for analysis. Ten high-power 

Physiological measurements. Starting at 0700, ruminal pH was recorded every minute during the last 2 days of each experimental week using a pH monitoring system and methodology previously described by A1Zahal et al. (2). Ruminal fluid samples were collected via the cannula at 1900 on the last day of each experimental week when we suspected ruminal pH to be depressed, on the basis of previous experimentation (29, 62), to temporally characterize how ruminal SCFA concentrations and osmolality respond to shifts in dietary grain. After collection, the ruminal fluid samples were immediately frozen at −80°C until analysis. Ruminal SCFA concentrations were quantified in duplicate by gas chromatography using methods previously described by Mutsvangwa et al. (42). The osmality of the rumen fluid samples was determined by freezing point depression using an automatic osmometer (31).

Rumen papillae biopsies. Rumen papillae were biopsied from the ventral sac at the end of experimental weeks 0, 1, 3, 4, and 6 (0700 after 48 h of continuous ruminal pH recording), as described by Kelly et al. (28). In brief, the reticulorumen contents were partially evacuated to facilitate the retraction of the ventral sac. Approximately 150 mg of rumen papillae were collected from exclusive sites in the ventral sac during each biopsy and washed in ice-cold PBS (pH = 7.4, 1X) 20 times prior to immediate fixation or freezing.

Microscopy. Five papillae were prepared for light microscopy histomorphometric analysis using methods previously described by Odongo et al. (47). Formalin-fixed, paraffin-embedded papillae were sectioned at a 4-µm thickness, stained with hematoxylin and eosin, and mounted for analysis with a microscope. Within the biopsy area, five fields were measured using a 20X objective lens, and two images were captured per papillae for a total of four measurements per papillae and 20 for each animal biopsy. Two separate estimates were made for each preplanned parameter on all layers of the SSE. Image Pro Plus software (Media Cybernetics, Bethesda, MD, USA) was used to measure predefined criteria (a review of the criteria for epithelial layer measurements has been summarized in the Supplemental Material 1).

In addition to measurements of cell layers, lesion scores on each papillae were also quantified using the Olympus BX60 light microscope and a 10X objective lens. A score of 1 indicated nil to minor lesions, a score of 5 indicated minor lesions with corneum sloughing, and a score of 9 indicated severe deep lesions with large amounts of corneum sloughing. Five papillae per cow per week were examined for lesion scoring (36).

Additional papillae were prepared for electron microscopy using methodology reported by Graham and Simmons (21). Washed papillae were immediately fixed in 2% glutaraldehyde for 24 h, postfixed for 1 h in 1% osmium, and dehydrated in a graded series of ethanol solutions. For scanning electron microscopy (SEM), the papillae were subjected to critical-point drying using liquid CO2 as the medium, mounted and coated with gold. Samples were then examined using SEM (Hitachi S-570, Hitachi Technologies, Tokyo, Japan). For transmission electron microscopy (TEM), dehydrated samples were then placed in a mixture of Spurr resin (60) and acetone (1:1) for 30 min, followed by 2 h in 100% resin with changes every hour. Samples were then embedded in resin and sectioned using a diamond knife. Sections were collected on 600-mesh copper grids, stained with lead citrate, and examined using a Jeol transmission electron microscope (Jeol 2010, Jeol USA, Peabody, MA).

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containing resin were placed in molds and polymerized at 70°C for 3 h to 3 days. Semithin (0.25–0.5 μm) sections were cut with glass knives and stained with 1% toluidine blue-O in 1% sodium borate. Ultrathin (70–90 nm) sections were cut with a diamond knife, stained with methanolic uranyl acetate followed by lead citrate, and examined using TEM (Philips Model EM 208S; Feico, Hillsboro, OR, USA).

**RNA isolation and quality assessment.** Rumen papillae that were immediately frozen in liquid nitrogen were then stored at −80°C until RNA isolation. Total RNA was isolated using an RNeasy midi kit (Qiagen, Missisauga, Canada), treated with DNase (Invitrogen, Burlington, Canada), and assessed for quality using a Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and the RNA 6000 Nano kit (Caliper Life Sciences, Mountain View, CA). The RNA Integrity Number of samples taken for microarray and quantitative real-time (qRT-PCR) analysis were 9.88 ± 0.21 and 8.75 ± 0.20, respectively.

**Microarray screen.** Microarrays were used to screen the expression patterns in rumen papillae from each cow (n = 4) for candidate genes associated with IGF, EGF, and desmosomes during weeks 0, 1, and 3 (12 microarrays in total). Hybridization of microarrays and data acquisition was carried out in the London Regional Genomics Facility (University of Western Ontario, London, Canada) using the 24K Affymetrix GeneChip Bovine Genome Arrays (Affymetrix, Santa Clara, CA). A complete description of the microarray hybridization protocol can be found in the Supplemental Material 1 and the GEO repository (GSE17849). The effect of dietary treatment on gene expression (relative mRNA content) was evaluated after subjecting the data to the GC Robust Multichip Analysis (GCRMA) preprocessor in Partek (Partek, St. Charles, MO; http://www.partek.com/) at the University of Kentucky Microarray Core Facility. Week 0 was compared with weeks 1 and 3 by ANOVA and then a means separation test was used to uncover differentially expressed genes (P < 0.05; fold change greater than ± 1.5) using Partek.

**qRT-PCR.** Candidate genes from our selected gene families that met our criteria were validated using qRT-PCR on all biopsied rumen papillae. After RNA isolation and quality assessment, samples were reverse transcribed before iTaq SYBR Green (Bio-Rad Laboratories, Mississauga, Ontario, Canada) qRT-PCR analysis in triplicate, using an ABI Prism 7000 (Applied Biosystems, Streetsville, Ontario, Canada). When possible, exon-spanning primers were designed for target genes using Primer Express 3.0 (Applied Biosystems). Dissociation curves were generated at the end of amplification to verify the presence of a single product. The efficiency of qRT-PCR amplification for each gene was calculated using the standard curve method (R², standard curve slope, and primer efficiencies of primers are presented in Table 1). Three common bovine housekeeping genes were originally tested for all samples prior to qRT-PCR analysis, and GAPD and beta actin were determined to be suitable housekeeping genes for this study [standard deviation = 0.12, ± Ct (cycle threshold); coefficient of variation = 0.64, %Ct] (52).

**Calculations and statistical analyses.** Values from the 2 days of ruminal pH recordings were averaged for each experimental week before analysis. A mixed-model procedure with repeated measures (67) in Statistical Analysis System (56) was used to contrast ruminal pH, ruminal SCFAs, and osmolality data between experimental weeks and diets. The model included the fixed effect of time and the random effect of the cow, and then the best-fitting covariance structure for each variable was determined using the Akaike criterion. To analyze mRNA expression, normalized qRT-PCR data were transformed to obtain a perfect mean of 1.0 at week 0. The abundance of genes was calculated using the inverse of qRT-PCR efficiency raised to Delta Ct (10, 57). For the temporal gene expression analysis, normalized qRT-PCR data were presented as fold-change relative to the first baseline HF measurement (week 0). Gene expression data were also analyzed using the same mixed model procedure with repeated measures to determine significance. For all statistical analysis, the α priori significance for difference between means was P < 0.05.

**RESULTS**

**Continuous ruminal pH, SCFA concentrations, and osmolality.** As expected, the mean, maximum, and minimum daily ruminal pH values were higher during the HF period compared with the HG period (P < 0.01) (Fig. 1). *Week 1* of the HG period was marked by the lowest daily ruminal pH mean (5.86 ± 0.05), minimum (5.26 ± 0.06), and maximum (6.40 ± 0.02; Fig. 1). Ruminal pH during weeks 2 and 3 of the HG diet increased to HF levels, and ultimately increased by week 4 to equal the mean and maximum ruminal pH baseline measure, while on the baseline diet (Fig. 1). As presented by Steele et al. (61), SARA was diagnosed during *week 1* of the HG period, as ruminal pH was depressed below 5.6 for 4.6 ± 1.4 h daily and below 6.0 for 14.9 ± 5.2 h/day. During *weeks 2 and 3* of the HG period, no time was spent below pH 5.6 and, therefore, SARA could not be diagnosed.

The concentration of SCFAs and the osmolality in rumen fluid were higher (P < 0.01) during the HF period compared with the HF period (Supplemental Material 1, Supplemental Table S1). As presented by Steele et al. (61), ruminal fluid SCFA concentrations were highest during the first week of the HG diet (99.89 ± 5.56 mmol) but gradually declined (P < 0.01) during the next 2 wk. Ruminal butyrate had the largest increase (330%) from baseline to *week 1* before decreasing (P < 0.01) but remained elevated compared with baseline

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**Table 1. Primers for quantitative real time PCR**

<table>
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<tr>
<th>Gene Name</th>
<th>Gene ID</th>
<th>Primers*</th>
<th>Amplicon Size, bp</th>
<th>Eff%**</th>
</tr>
</thead>
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<td>IGF binding protein 3 (IGFBP3)***</td>
<td>NM_001075549</td>
<td>F855-CCGACAGAAGAGCTGTTTACA</td>
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<tr>
<td>IGF binding protein 5 (IGFBP5)</td>
<td>BC102850</td>
<td>R936-5ATCCCACACAGAGCAAGAAC</td>
<td>63</td>
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<tr>
<td>IGF binding protein 6 (IGFBP6)</td>
<td>NM_001105613</td>
<td>F701-CTACAGAGAAGGCACTGCGAGAC</td>
<td>84</td>
<td>91.5</td>
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<tr>
<td>Desmoglein 1 (DSG1)</td>
<td>NM_001013004</td>
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<td>121</td>
<td>88.1</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate</td>
<td>NM_177497</td>
<td>F440-GGCAGAGACGAAACGAGGAACT</td>
<td>89</td>
<td>91.5</td>
</tr>
<tr>
<td>dehydrogenase (GAPD)******</td>
<td>BF041965</td>
<td>R527-6GAGCACTACGCGACTGCTGT</td>
<td>103</td>
<td>90.0</td>
</tr>
</tbody>
</table>

*Exon junctions underlined **The PCR efficiency was calculated as follows: E = 1 + 10^(-1/slope) × 100; the slope was obtained using the 5-point standard curve, with a minimum R² of 0.99. ***IGFBP3 primers previously used by Loor (38). ****GAPD primers previously used as housekeeping genes in bovine liver (26). *****ACTB primers previously used as housekeeping genes in bovine liver and muscle (23).
Ruminal fluid osmolality shared a similar pattern with the ruminal SCFA results. Osmolality was also highest during week 1 (327 ± 11 mOsm/kg; P < 0.05); however, it returned to levels not significantly different from baseline once the cattle returned to the HF diet.

Histology, morphology, and ultrastructure of rumen papillae. Representative light micrographs of rumen papillae cross sections from the HF and HG diets are shown in Fig. 2, A and B. Cross sections of rumen papillae viewed under the light microscope revealed no significant difference in the width of stratum corneum between the HF and HG diet, regardless of period. In contrast, the thickness of the strata granulosum, basale, and spinosum, as well as the entire SSE thickness in the biopsied rumen papillae, was reduced (P < 0.01) during the HG diet. During the interval between the baseline week and week 1 of the HG diet, the thickness of the stratum granulosum, as well as the sum of the stratum basale and spinosum, was reduced from 29.5 ± 2.5 µm and 129.4 ± 8.0 µm to 26.5 ± 0.6 µm and 117.2 ± 3.3 µm, respectively. The reintroduction of the HF diet was marked by increased (P < 0.01) thickness of all living strata to levels not significantly different from baseline. Lesion scoring was not significantly different between treatments; however, week 1 of the HG period was marked by the highest ruminal lesion score of 5 compared with all other weeks. Lesion scores increased (P = 0.02) from baseline to week 1 of the HG diet, yet declined to levels not significantly different from baseline by week 3 of the HG diet.

SEM micrographs of rumen papillae from all cattle during the HF period revealed deep ridges and indentations on the surface (Fig. 2C). The deep ridges were rich in morphologically heterogeneous microflora, including bacteria and protozoa (Supplemental Material 1, Supplemental Fig. S1, A and B). During the first week of the HG diet, the ridges became shallow (Fig. 2D), concomitant with a reduction in microbial colonization (Supplemental Material 1, Supplemental Fig. S1,
F and G). At high magnification, individual granular keratinized squamous cells of the stratum corneum were evident. Desquamation of the dead keratinized cells was apparent throughout the surface of the papillae in both diets; however, it was most striking during the HG diet in all cattle, especially during week 1 when SARA occurred.

Transmission electron micrographs of rumen papillae cross sections during the HF diet displayed a higher degree of intracellular organization at all levels with few intracellular spaces (Fig. 3A). Cells were strongly anchored to one another with tight cellular junctions, especially desmosomes. The stratum corneum was compact with few signs of delamination. The living strata displayed very tight, well-defined, zona occludens and desmosomes (Supplemental Material 1, Supplemental Fig. S1, C–E). Few transition cells were found between the stratum corneum and stratum granulosum. The cells of the stratum basale and stratum spinosum were cuboidal or short and columnar with long axes perpendicular to the basal lamina. These cells possessed large ovoid nuclei and numerous mitochondria, ribosomes, endoplasmic reticulum, and Golgi bodies, as well as large vesicles, predominantly at the apical part of the cell.

During the HG diet, the ultrastructure of the ruminal SSE, as viewed by TEM, was dramatically altered as large spaces between levels of strata containing large amounts of bacteria were evident. In addition, cells in all strata below the stratum corneum appeared to have an accelerated migratory state (Fig. 3B). Extensive sloughing of the stratum corneum was observed throughout the epithelial surface, especially during the first week of the HG diet when SARA occurred. High-magnification SEM revealed cytoplasmic protrusions with a microvilli-like appearance from the corn cells of the corneum (Fig. 4A). The corn cell cytoplasmic protrusions were longer and not as densely spaced during the HG diet compared with the HF diet. The demarcation of the different strata became diffuse, as cells from the stratum basale migrated luminally at an increased

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**Fig. 3.** Comparison of rumen papillae ultrastructure during the high-forage (week 0) and high-grain (week 1) periods. A: Transmission electron microscopy (TEM) of papillae cross section during the high-forage period (scale bar = 6 μm). B: TEM of papillae cross section during the high-grain period (scale bar = 6 μm).

**Fig. 4.** Ultrastructure of the rumen epithelium during high-grain feeding using SEM and TEM. A: SEM of rumen papillae surface. Note the extended cytoplasmic protrusions from the horn cell (CP) (scale bar = 3 μm). B: TEM of stratum granulosum cell with intact nuclei (N) and keratohyaline-like granules (KG) bordering the stratum corneum (scale bar = 3 μm). C: TEM of stratum basale cell rich in mitochondria (M) with diffuse junctions with large extracellular space (ES) between neighboring cells (scale bar = 3 μm).
rate. The adhesion between cells of the stratum corneum and stratum granulosum appeared to be compromised, as evidenced by large gaps between cells. Cell junctions, especially desmosomes, became indistinct during the HG period, and there was little indication that granulosum cells were forming a tight barrier. Granular cells bordering the stratum corneum had a distinct flattened shape and contained large keratohyaline granules (Fig. 4B). Cells of the stratum basale became more pyramidal in shape and displayed the largest intracellular spaces, some approaching 3 μm (Fig. 4C, Supplemental Material 1, Supplemental Fig. S1, H–J).

Microarray screen and qRT-PCR. On the basis of preliminary candidate gene selection by microarray (Supplemental Material 2), the cadherin desmoglein 1 (DSG1) and IGFBP 3, 5, and 6 met our predetermined criteria (P < 0.05, fold change more than ± 1.5) and were validated by qRT-PCR. DSG1 mRNA expression was highest (P < 0.01) during the HF diet compared with the HG diet (Fig. 5A). The expression was downregulated by 0.25 ± 0.03-fold from baseline to week 3. Once the cattle were switched back to the HF diet, expression increased to 3.57 ± 0.55-fold compared with baseline and remained elevated compared with the HG diet for the remainder of the experiment.

Differential mRNA expression was observed for all three IGFBPs during the HG diet (Fig. 5B). IGFBP5 mRNA expression was upregulated during weeks 1 and 3 from 1.60 ± 0.16-fold to 1.79 ± 0.32-fold, respectively. In contrast, IGFBP3 mRNA expression was downregulated by 0.53 ± 0.05-fold during week 1 of the HG diet and 0.70 ± 0.03-fold by week 3. IGFBP6 mRNA expression followed a similar expression pattern to IGFBP3 as mRNA levels were depressed 0.70 ± 0.07-fold and 0.56 ± 0.05-fold during weeks 1 and 3 of the HG diet, respectively. The mRNA expression of all three IGFBPs during weeks 4 and 6 of the HF diet was not significantly different from the baseline period (week 0, HF).

**DISCUSSION**

The objectives of this study were to characterize the structural adaptations of the ruminal SSE during SARA and to identify prospective molecular mechanisms orchestrating these changes. Hence, it was imperative that we characterize rumen papillae histology, ultrastructure and, concurrently, mRNA expression of key genes at different stages of adaptation to the HG diet. In this study, SARA was induced and naturally mitigated by the second and third weeks of HG feeding. These results agree with the time-dependent increases in ruminal pH after prolonged adaptation to starch previously reported in dairy cattle (40). It has recently been demonstrated in sheep that the epithelial capacity for apical uptake of SCFAs is a key determinant for intraruminal pH and susceptibility to SARA (51). To date, enhanced absorption of SCFA is thought to be achieved by increasing the epithelial surface area coupled to increased activity of ion exchangers (15, 37). Our findings suggest that more factors are involved in ruminal SSE adaptation, both structural and functional, as evidenced by microscopic and qRT-PCR analyses. The cellular and molecular adaptations of the SSE involved in elevating ruminal pH during the final weeks of the HG diet in this study may be useful in the prevention and treatment of ruminal acidosis induced by rapid shifts in dietary carbohydrates.

Rumen SSE proliferation (54) and metabolism (28) can dynamically change throughout the day, but it is unknown how quickly microarchitecture can be modified. As demonstrated in this study, a dramatic adaptation of the ruminal SSE was observed after only 1 wk of feeding the HG diet. Histological and ultrastructural micrographs of the ruminal epithelium indicated a deterioration of cellular junctions and large spaces between cells, thus providing opportunity for microbial infiltration from rumen to blood. Stratum granulosum thickness was reduced, and it was apparent from TEM images that the degree of cellular adhesion was decreased as the number of desmosomes diminished. Our results are in contrast to a study conducted in sheep that demonstrated that high-energy diets have a positive effect on barrier function of the rumen epithelium (37). However, unlike many studies investigating ruminal SSE adaptation to high-grain diets, we found no accumulation of the stratum corneum, which may be causing this effect in other experimental models. It is also important to note that alterations in the normal expression pattern of desmosomal cadherins can result in compromised epidermal structure and function (25). In this study, desmosomal cadherin DSG1 was downregulated during high-grain feeding. DSG1 is known to be highly expressed in most SSE, particularly in the stratum granulosum, unlike DSG2, which is expressed at lower levels.
upon microarray data (Supplemental Material 2). Most EGF is production of EGF or its binding protein in rumen papillae based on its role in regulating proliferation and morphogenesis in vivo. As EGF and IGF, have been shown to increase proliferation of cells in the ruminal epithelium. This suggests that these growth promoters, such as SCFAs, may be involved in modulating cellular regulatory mechanisms that can increase or decrease the rate of mitosis and cellular differentiation according to ruminal conditions. Goodlad (18) suggested that the ruminal SSE possesses self-regulatory mechanisms that can increase or decrease the rate of mitosis and cellular differentiation according to ruminal SCFAs, yet the molecular basis of this putative cellular regulatory mechanism remains unknown. Growth promoters, such as EGF and IGF, have been shown to increase proliferation of rumen epithelial cells in vitro (4), and their gene families may be regulating proliferation and morphogenesis in vivo.

In this study, we found no evidence of differential expression of EGF or its binding protein in rumen papillae based upon microarray data (Supplemental Material 2). Most EGF is produced in the parotid gland and supplied to the rumen via saliva, while limited expression has been detected in rumen tissue (48). Since HG diets typically lack physically effective fiber, which supports saliva production (7), it is highly unlikely that EGF plays an important role in the adaptive response of the ruminal SSE during a grain challenge.

In contrast to EGF, the IGF family has received considerable attention as IGF-1 plasma concentrations are correlated with rumen papillae growth, when ruminants are fed higher levels of butyrate (19) and energy (59). IGFBPs have been shown to regulate IGF-stimulated proliferative events in epithelial tissue (14) and have been proposed as playing a role in bovine mammary epithelial differentiation (39). Their expression can be independent of IGF-1 concentrations as SCFA concentrations can regulate the expression of IGFBPs in the intestinal epithelium (55). Furthermore, studies of colonic cell culture with varying SCFA concentrations have shown that SCFA can alter the expression of IGFBPs, thereby triggering cellular proliferation (43).

In this study, we found that the grain challenge downregulated IGFBP3 and IGFBP6 and upregulated IGFBP5. Of the IGFBPs, IGFBP3, and IGFBP5 have been extensively characterized and are thought to modulate IGF-1 cellular events in an opposing fashion (6). In the case of IGFBP5, it is known to potentiate IGF-1 effects, which may encourage proliferation in the rumen epithelium when upregulated (14). Since IGFBP3 is thought to block IGF-1 cellular events, its downregulation, as seen in this study, would encourage tissue growth (6). We also noted that few transition cells were found between the stratum corneum and granulosum in our histological analysis, which suggests a slow apoptotic rate of granular cells. Interestingly, the SCFA, butyrate, downregulates the expression of IGFBP3, blocking apoptosis in intestinal epithelial cells (55). This finding agrees with Mentschel et al. (41), who reported that intraruminal butyrate infusions induced papillae growth by decreasing apoptosis in the SSE. Since ruminal butyrate concentrations were elevated during the HG diet in the present study (Supplemental Material 1, Supplemental Table S1), it is plausible that butyrate can trigger the downregulation of IGFBP3 in the ruminal SSE, thus blocking apoptosis and increasing growth. In contrast to IGFBP3 and IGFBP5, IGFBP6 binds IGF-2 preferentially over IGF-1. In cancer cells, IGFBP6 functions as an inhibitor of IGF-2 cellular events; therefore, it is possible that it has a similar role in rumen papillae, as it is downregulated during high-grain feeding (3). Similarities in our expression profile compared with previous experiments in intestinal cells support the hypothesis that shifts in ruminal SCFA concentrations during grain feeding may regulate the expression of IGFBPs, thereby controlling ruminal SSE proliferation and differentiation.

### Perspectives and Significance

Our findings provide the first detailed characterization of ruminal SSE adaptation during SARA. This study introduces new information to describe epithelial adaption during grain feeding, including increased detail concerning the proliferation, differentiation, and migration of the cells of the SSE in response to increased fermentable dietary carbohydrates. Our findings suggest that HG diets and SARA may be associated with increased permeability through reduced organization and...
thickness of the ruminal SSE and reduced intercellular adhesion between cells of stratum granulosum. Additionally, HG diets induce accelerated cellular migration and postmitotic aging during SARA. Together, these changes are likely responsible for the cumulative effect commonly known as parakeratosis. Finally, we also identified molecular markers (DSG1, IGFBP3, IGFBP5, and IGFBP6) that may play a pivotal role in signaling the adaptive response of the SSE during a HG diet. To advance our knowledge, it is imperative to study the expression of candidate genes at the protein level and at different time points relative to feeding. Further research is necessary to enhance our understanding of the cellular and molecular adaptive mechanisms of the SSE during grain challenges or SARA to develop technologies to attenuate the detrimental welfare and productive impact that acidosis imposes on ruminants.

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DISCLOSURES

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