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The relationship between rumen acidosis resistance and expression of genes involved in regulation of intracellular pH and butyrate metabolism of ruminal epithelial cells in steers

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ABSTRACT

Past research has focused on the prevention and management of subacute rumen acidosis by manipulating the ration; however, the severity of acidosis varies even among animals fed a common high-grain diet. The objectives of this study were to compare the ruminal volatile fatty acid (VFA) profile and expression of genes involved in the metabolism of butyrate, the VFA most extensively metabolized by the ruminal epithelium, and intracellular pH regulation in ruminal epithelial cells between acidosis-resistant (AR) and acidosis-susceptible (AS) steers. Acidosis indexes (area per day under pH 5.8 divided by dry matter intake) were measured for 17 steers fed a common high-grain diet, and the 3 steers with the lowest (1.4 ± 1.2 pH·min/kg) and the 3 with the highest values (23.9 ± 7.4 pH·min/kg) were classified as AR and AS, respectively, and used in the subsequent study. The steers were force-fed a diet containing 85% grain at 60% of the expected daily intake (5.8 ± 0.8 and 5.6 ± 0.6 kg for AR and AS, respectively) within 30 min. Mean ruminal pH over the postprandial 6-h period was higher for AR compared with AS (6.02 vs. 5.55), and mean total VFA concentration was 74% for AR compared with AS (122 vs. 164 m*M*). Molar proportion of butyrate in the ruminal fluid was 139% higher for AR compared with AS (17.5 vs. 7.33 mol/100 mol of VFA). Expression of monocarboxylate cotransporter isoform 1, sodium hydrogen exchanger isoforms 1 and 2, and anion exchangers (downregulated in adenoma and putative anion exchanger, isoform 1) did not differ between AR and AS steers. However, expression of sodium hydrogen exchanger isoform 3, which imports Na^+ to the epithelial cell and exports H^+ to the rumen, was 176% higher in AR steers than in AS steers. Higher ruminal pH for AR might be partly due to a faster rate of VFA absorption, lower VFA production, or both.

Key words: rumen acidosis, volatile fatty acid absorption, volatile fatty acid metabolism, gene expression

INTRODUCTION

Subacute rumen acidosis is a metabolic disorder that greatly affects the dairy industry, causing great economic losses for the producer (Stone, 2004). This disorder usually is not associated with acute clinical signs (Nagaraja and Lechtenberg, 2007), making it difficult to define and detect on farms. However, SARA is associated with liver abscesses (Nagaraja and Lechtenberg, 2007), laminitis (Nocek, 1997), decreased appetite, and depressed milk fat (Kleen et al., 2003), all of which may result in substantial economic losses for the dairy industry. The majority of past research has focused on nutritional management, such as fermentability of the diet and physical effectiveness of fiber. Although nutritional management practices can reduce the incidence of SARA, some animals are more susceptible to the effects of a high-grain diet than others. Brown et al. (2000) showed that the severity of acidosis varies among steers fed a common high-grain diet, which may hold true for lactating dairy cows fed a high-grain diet.

Ruminal pH is maintained by a balance between acid production by microbes in the rumen and its removal by absorption through the ruminal epithelial cells, neutralization with salivary buffers, and passage to the lower digestive tracts (Allen, 1997). Accumulation of VFA in the rumen causes pH depression and SARA. Allen (1997) estimated that approximately 37% of protons are neutralized in the rumen by salivary buffers, whereas about 7% of protons disappear from the rumen by passage to the lower digestive tracts, leaving more than one-half of the protons to be removed by absorption through the ruminal epithelial cells or neutralization by buffers excreted by the epithelial cells. Penner et al. (2009a) demonstrated that ruminal epithelial cells from acidosis-resistant sheep had a greater capability for uptake of VFA in vitro, indicating that the rate of VFA absorption may partly affect the extent of resistance to rumen acidosis in vivo.

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Therefore, the relationship between ruminal pH and expression of genes coding VFA transporters and a key enzyme involved in energy metabolism in ruminal epithelial cells warrants investigation. The objective of this study was to determine whether differences existed in the expression of genes involved in VFA absorption and energy metabolism in ruminal epithelial cells between acidosis-resistant (**AR**) and acidosis-susceptible (**AS**) animals.

MATERIALS AND METHODS

Animals used in this study were cared for in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada). All procedures were approved by the Animal Care Committee of the University of Alberta Research Centre.

Screening Study

Seventeen ruminally cannulated steers (539 ± 49.5 kg; mean ± SE) were used as a model for ruminants experiencing SARA for this study. The steers were housed in individual pens bedded with wood shavings and fed a diet consisting of 85% grain (Table 1) ad libitum. Ruminal pH was measured in the ventral sac every 30 s continuously for 3 d, using the pH measurement system evaluated by Penner et al. (2009b). Minimum, mean, and maximum pH were determined, as well as duration and area below pH 5.8. These data were used to determine the acidosis index (area under pH 5.8 divided by DMI; Penner et al., 2009c), and the 3 steers with the lowest values and the 3 steers with the highest values were selected as AR and AS animals, respectively, and were used in the subsequent study on the following day.

Sample Collection and Analysis

Three AR and 3 AS steers were force-fed a common diet (Table 1) at 60% of their average DMI over the 3 d immediately before the study; all leftover rations were placed into the rumen through ruminal cannulas at 30 min after feeding to induce SARA. Data and samples were collected during the 6-h postprandial period.

Ruminal pH Measurement. Ruminal pH was measured in the ventral sac every 30 s for the 6-h data collection period, using the system evaluated by Penner et al. (2009b). Minimum, mean, and maximum pH values, duration and area below pH 5.8, and acidosis index (area <pH 5.8/DMI) were determined for each steer.

Ruminal Fluid Collection. Ruminal fluid was collected from 5 locations in the rumen immediately before feeding and every 2 h for the subsequent 6-h period, and the fluid was combined and strained through a perforated screen (Peetex, Sefar Canada Inc., Scarborough, ON, Canada; pore size = 355 μm). Samples were centrifuged at 3,000 × *g* for 20 min at 4°C, and the supernatant was stored at −20°C for subsequent analysis.

Ruminal fluid samples were analyzed for VFA profile using gas chromatography. Samples were injected by an autosampler (Model 8200; Varian Inc., Walnut Creek, CA) into a Stabilwax-DA column (30 m × 0.53 mm i.d. × 0.5 μm film thickness, Restek Corporation, Bellefonte, PA) on a Varian gas chromatograph (Model 3400). Samples were run at a split vent flow of 20 mL/min with a column temperature of 90°C for 0.1 min, and then increased to 170°C at a rate of 10°C/min and held for 2 min at 170°C. The injector temperature was 170°C, and the detector temperature was 190°C. Peak integration was evaluated using Galaxie Software (Varian Inc.). All samples were assayed in duplicate.

Blood Collection. Blood was collected from the jugular vein through a catheter immediately before feeding and every 2 h for the subsequent 6-h period into tubes containing sodium heparin (Fisher Scientific Company, Nepean, ON, Canada). Blood samples were centrifuged at 3,000 × *g* at 4°C for 20 min immediately after collection, and plasma was harvested and stored at −20°C until analysis.

Plasma samples were analyzed for glucose concentration using a glucose oxidase/peroxidase enzyme (Sigma, St. Louis, MO) and dianisidine dihydrochloride (Sigma) procedure. Absorbance was determined using a SpectraMax 190 plate reader (Molecular Devices Corp., Sunnyvale, CA) at a wavelength of 450 nm. Plasma BHBA was quantified by oxidizing BHBA to acetoacetate using 3-hydroxybutyrate dehydrogenase (Roche,

Table 1. Ingredient and chemical composition of the diet

Item	Amount, % of DM
Ingredient	
Sun-cured alfalfa pellet	10.0
Mineral and vitamin mix[1]	5.0
Barley grain, dry rolled	56.7
Oats grain, dry rolled	28.3
Nutrient composition	
DM	92.2
Ash	6.5
CP	17.6
NDF	28.7
Starch	38.6

[1]Contained 6.00% Ca, 0.49% P, 1.60% Na, 0.65% Mg, 0.65% S, 0.20% K, 13.0 mg/kg of I, 220.0 mg/kg of Fe, 242.0 mg/kg of Cu, 815.0 mg/kg of Mn, 11.0 mg/kg of Co, 1,220.0 mg/kg of Zn, 6.00 mg/kg of Se, 440.0 mg/kg of monensin Na, 90 kIU/kg of vitamin A, 13.3 kIU/kg of vitamin D_3, 0.40 kIU/kg of vitamin E.

Mississauga, ON, Canada) and measuring the reduction of NAD^+ to NADH in a 0.2 *M* Tris buffer/NAD solution (Sigma) using a SpectraMax 190 plate reader at a wavelength of 340 nm. Commercial kits were used to determine concentrations of plasma NEFA (Wako Chemicals USA Inc., Richmond, VA) and insulin (Coat-a-Count Kit Diagnostic Products Corporation, Los Angeles, CA).

Ruminal Papillae Collection. Steers were treated with Liquamycin LA-200 (2 mg/100 kg of BW; Pfizer Animal Health, New York, NY) 1 d before the sample collection date. Approximately 30 ruminal papillae were biopsied immediately before feeding, and an additional 30 papillae were biopsied every 2 h for the subsequent 6-h period. The papillae were rinsed with PBS and stored at −20°C in RNA-Later solution (Ambion Inc., Foster City, CA) until analysis.

RNA Extraction. Total RNA was extracted from the ruminal papillae using the Trizol extraction method (Invitrogen, Burlington, ON, Canada) as described by Chomczynski and Sacchi (1987) and purified using a Qiagen RNeasy MiniElute Cleanup Kit (Qiagen, Toronto, ON, Canada). The RNA concentration of the extract obtained was determined using a NanoDrop 2000C Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at absorbances of 260 and 280 nm. The 260/280 absorbance ratio of the samples was at least 1.93.

Reverse Transcription. The RNA samples were diluted to 100 ng/μL and subsequently treated with DNase I (Invitrogen, Carlsbad, CA) and RNase OUT (Invitrogen). First-strand cDNA was synthesized using Superscript II (Invitrogen).

Primer and Probe Design. Primers and probes were designed using Primer Express software (Applied Biosystems, Foster City, CA) and verified for specificity using the National Center for Biotechnology Information database. The target genes, housekeeping genes, primer and probe sequences, and National Center for Biotechnology Information accession numbers are shown in Table 2.

Quantitative Real-Time PCR. Genes encoding monocarboxylate cotransporter, isoform 1 (**MCT1**), downregulated in adenoma (**DRA**), putative anion transporter, isoform 1 (**PAT1**), sodium hydrogen exchanger, isoforms 1, 2, and 3 (**NHE1**, **NHE2**, **NHE3**, respectively), and Na^+/K^+ ATPase pump (see Table 2) were evaluated for their expression in ruminal epithelial cells via quantitative real-time PCR, using a TaqMan gene expression assay with The StepOne Plus Real-Time PCR System (Life Technologies, Carlsbad, CA). The program consisted of a 95°C preincubation for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. All samples were analyzed on 1 plate per gene, using 40 ng of cDNA per reaction. Samples before feeding were assayed in triplicate, whereas samples from 2, 4, and 6 h after feeding were analyzed in quadruplicate.

The expression of each targeted gene was evaluated using the comparative cycle threshold ($\mathbf{C_T}$) method and normalized using 3 housekeeping genes, ribosomal protein large, P0, β-actin, and GADPH, according to the method described by Vandesompele et al. (2002).

Statistical Analysis

Body weight, DMI, and pH data were analyzed using the PROC TTEST procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). The other data were analyzed using the PROC MIXED procedure of SAS (version 9.2; SAS Institute Inc.) with time as a repeated measure and animal as the experimental unit according to the model below, using the variance/covariance structure of best fit as described by the Akaike information criterion:

$$Y_{ij} = \mu + G_i + H_j + GH_{ij} + e_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean, G_i is the effect of *i*th group, H_j is the effect of *j*th time point, and e_{ij} is the error term.If significance was found, LSMEANS were determined and the PDIFF procedure was used with the Bonferroni correction to determine differences between means. The largest standard error values are reported where applicable. Significance was declared at $P < 0.05$, and trends were declared at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Screening Study

Mean ruminal pH, duration of pH below 5.8, and area of pH below 5.8 ranged from 5.44 to 6.13, 243 to 1,291 min, and 34 to 621 pH·min, respectively. The acidosis index for 17 steers ranged from 4.0 to 96.5 pH·min/kg.

No differences in DMI ($P > 0.10$; Table 3) were observed between the 3 steers classified as AR and the 3 steers classified as AS; however, mean pH was higher for AR steers compared with AS steers (6.01 vs. 5.51, respectively, $P = 0.01$), duration of pH below 5.8 was lower for AR steers compared with AS steers (481 vs. 1,130.3 min, $P = 0.03$), area of pH below 5.8 tended to be lower for AR steers compared with AS steers (157 vs. 535 pH·min/kg, $P = 0.01$), and acidosis index was lower for AR steers compared with AS steers (13.5 vs. 61.7 pH·min/kg, $P = 0.01$).

Table 2. Primer and probe sequences and National Center for Biotechnology Information accession numbers for quantitative real-time PCR analysis

Gene name	Category	Accession number	Primer and probe sequences
3-Hydroxy 3-methylglutaryl coenzyme A synthase isoform 2 (HMGCS2)	Ketogenesis	NM_001045883	Forward: CCT GCT GCA ATC ACT GTC ATG Reverse: TCT GTC CCG CCA CCT CTT C Probe: TTG CAG AGC CCT TTC
Sodium/potassium ATPase pump, α 1 (ATP1)	Energy metabolism	NM_001076798	Forward: CAT CTT CCT CAT CGG CAT CA Reverse: ACG GTG GCC AGC AAA CC Probe: TGT AGC CAA CGT GCC AG
Sodium/hydrogen antiporter, isoform 1 (NHE1)	VFA absorption	NM_174833	Forward: GAA AGA CAA GCT CAA CCG GTT T Reverse: GGA GCG CTC ACC GGC TAT Probe: AAG TAC GTG AAG AAG TGT CT
Sodium/hydrogen antiporter, isoform 2 (NHE2)	VFA absorption	XM_604493	Forward: TTG TGC GAT GAC CAT GAA TAA GT Reverse: TGA TGG TCG TGT AGG ATT TCT GA Probe: CGT GGA AGA GAA CGT G
Sodium/hydrogen antiporter, isoform 3 (NHE3)	VFA absorption	AJ131764.1	Forward: AGC CTT CGT GCT CCT GAC A Reverse: TGA CCC CTA TGG CCC TGT AC Probe: TGC TCT TCA TCT CCG
Putative anion transporter, isoform 1 (PAT1)	VFA absorption	BC_123616	Forward: GGG CAC TTC TTC GAT GCT TCT Reverse: GTC GTG GAC CGA GGC AAA Probe: TCA CCA AGC AGC ACC T
Downregulated in adenoma (DRA)	VFA absorption	NM_001083676.1	Forward: TGC ACA AAG GGC CAA GAA A Reverse: GCT GGC AAC CAA GAT GCT ATG Probe: TGC CTT CTC CTC CTT C
Monocarboxylate cotransporter, isoform 1 (MCT1)	VFA absorption	NM_001037319	Forward: CGC GGG ATT CTT TGG ATT T Reverse: GTC CAT CAG CGT TTC AAA CAG TAC Probe: TTT TGG GTG GCT CAG C
Ribosomal protein large, P0 (RPLP0)	Housekeeping gene	NM_001012682	Forward: AGG GCG TCC GCA ATG TT Reverse: CGA CGG TTG GGT AAC CAA TC Probe: CCA GCG TGT GCC TG
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Housekeeping gene	NM_001034034	Forward: TGC CGC CTG GAG AAA CC Reverse: CGC CTG CTT CAC CAC CTT Probe: CCA AGC GTG TGC CTG
β-Actin (ACTB)	Housekeeping gene	NM_173979.3	Forward: CCT GCG GCA TTC ACG AA Reverse: GCG GAT GTC GAC GTC ACA Probe: CTA CCT TCA ATT CCA TCA TG

Table 3. Dry matter intake and pH measurements of acidosis-susceptible (n = 3) and acidosis-resistant (n = 3) steers in the screening study

Variable	Resistant	Susceptible	SE	*P*-value
DMI, kg	10.4	9.0	1.29	0.45
Mean ruminal pH	6.01	5.51	0.105	0.01
Duration at pH <5.8, min	481	1,130	181.8	0.07
Area at pH <5.8, pH·min	157	535	105.4	0.01
Acidosis index, pH·min/kg	13.5	61.7	8.16	0.01

Ruminal pH and VFA Profile

No differences were observed in BW and DMI, including the force-fed rations, between AR and AS animals ($P > 0.10$; Table 4). However, the minimum (5.58 vs. 4.87; $P < 0.01$) and mean ruminal pH (6.05 vs. 5.59; $P < 0.05$) were higher for AR animals compared with AS animals, whereas maximum pH values were not different between the groups. Duration (224.7 vs. 80.0 min; $P < 0.01$) and area that pH was below 5.8 (133.0 vs. 7.67 pH·min; $P < 0.01$) were higher in AS animals. Acidosis index was higher in AS animals (23.9 vs.1.40 pH·min/kg; $P < 0.05$).

Total VFA concentration was lower for AR animals compared with AS animals (122 vs. 164 m*M*; $P < 0.01$; Table 5). Molar proportion of propionate in ruminal fluid was lower in AR steers compared with AS steers (21.2 vs. 37.5 mol/100 mol; $P = 0.01$), whereas concentrations of acetate (56.7 vs. 49.9 mol/100 mol; $P = 0.05$) and butyrate (17.5 vs. 7.33 mol/100 mol of VFA; $P < 0.01$) were higher for AR steers. No differences in isobutyrate, isovalerate, valerate, or caproate concentrations were observed between AR and AS animals ($P > 0.10$).

We observed significant differences in ruminal pH and the acidosis index between AR and AS in this study, although ruminal pH was similar before feeding (Figure 1). Because we enforced similar DMI between AR and AS animals and both groups of animals were fed a common diet, the difference in ruminal pH cannot be attributed to dietary factors. Total VFA concentration in the rumen was lower for AR animals compared with AS animals, although, like pH, ruminal VFA concentration was similar before feeding (Figure 2). As such, the lower postprandial ruminal pH for AR steers was likely due to lower VFA production, faster VFA absorption, or a combination of both factors. Although neutralization by salivary buffers and passage to the lower digestive tract also contribute to proton removal from the rumen, these factors were not measured in the present study. Allen (1997) estimated that approximately 53% of protons are removed from the rumen by VFA absorption, whereas about 37% are removed because of neutralization by salivary buffers, and 7% of proton removal is due to gut passage of VFA. We cannot exclude the possibility that the other acid removal pathways were different between AR and AS animals. However, absorption accounts for acid removal from the rumen to the greatest extent, and the expected difference in acid absorption between AR and AS animals warrants further investigation.

A marked increase in molar proportion of butyrate was observed for AR steers, whereas the sum of molar proportion of acetate and propionate was higher for AS steers compared with AR steers (87.4 vs. 77.9%; $P = 0.01$). Greater butyrate production may have at least partly contributed to the higher pH observed in AR steers because stoichiometric equations indicated that

Table 4. Comparison of BW, DMI, and pH measurements between acidosis-resistant and acidosis-susceptible steers

Variable	Resistant	Susceptible	SE	*P*-value
BW, kg	515	499	44.5	0.75
DMI, kg	5.84	5.63	0.446	0.72
Ruminal pH				
Nadir	5.58	4.87	0.114	<0.01
Mean	6.05	5.59	0.104	0.01
Maximum	6.55	6.47	0.225	0.79
SD	0.25	0.55	0.092	0.05
Duration at <pH 5.8, min	80	225	18.2	<0.01
Area at <pH 5.8, pH·min	8	133	20.1	<0.01
Acidosis index, pH·min/kg	1.4	23.9	4.28	0.03

Table 5. Comparison of ruminal VFA profile between acidosis-resistant and acidosis-susceptible steers

Variable	Resistant	Susceptible	SE	*P*-value
Total VFA, m*M*	122	164	5.3	<0.01
Acetate, mol/100 mol	56.7	49.9	1.68	0.05
Propionate, mol/100 mol	21.2	37.5	2.60	0.01
Isobutyrate, mol/100 mol	0.76	0.70	0.123	0.71
Butyrate, mol/100 mol	17.5	7.3	1.22	<0.01
Isovalerate, mol/100 mol	1.61	0.96	0.259	0.15
Valerate, mol/100 mol	1.96	2.30	0.221	0.34
Caproate, mol/100 mol	0.31	1.32	0.364	0.12

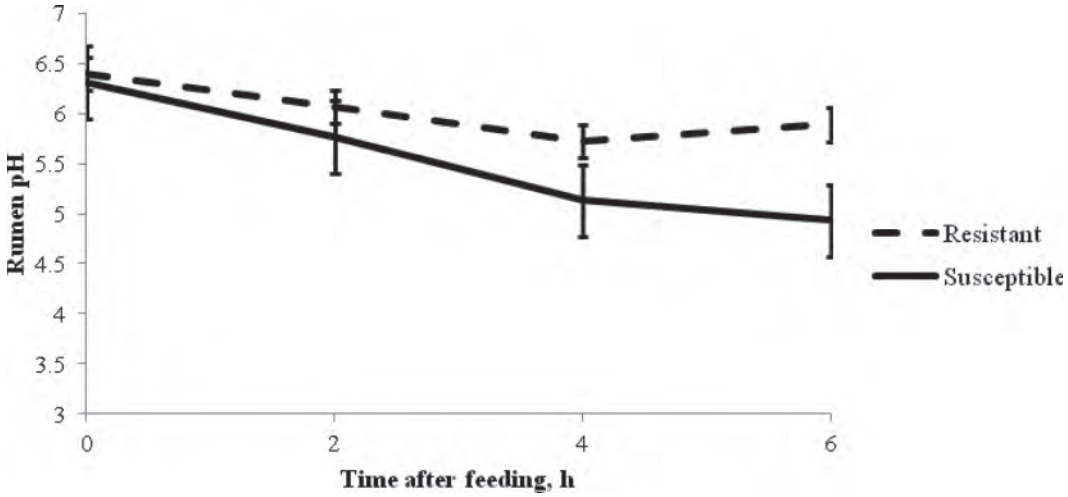

Figure 1. Comparison of ruminal pH between acidosis-resistant and acidosis-susceptible steers after feeding. Group effect, $P < 0.05$; hour effect, $P < 0.01$; group × hour interaction, $P = 0.02$.

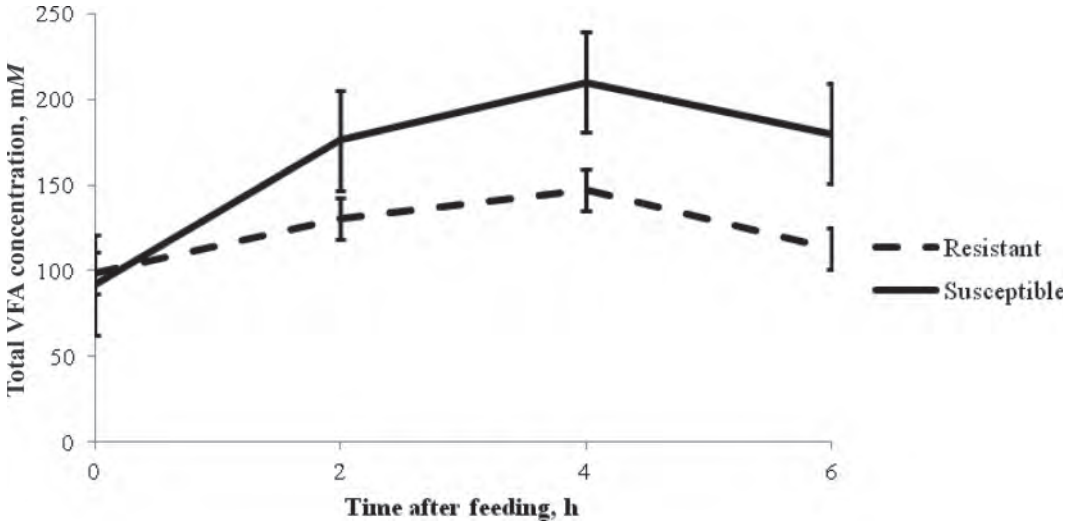

Figure 2. Comparison of total rumen VFA concentration between acidosis-resistant and acidosis-susceptible animals after feeding. Group effect, $P = 0.005$; hour effect, $P < 0.001$; group × hour interaction, $P = 0.02$.

Table 6. Comparison of mRNA abundance of genes involved in intracellular pH regulation and VFA metabolism between acidosis-resistant and acidosis-susceptible steers

Gene	Resistant	Susceptible	SE	*P*-value
MCT1	1.67	1.25	0.441	0.53
PAT1	1.64	1.48	0.353	0.77
DRA	2.42	1.46	0.437	0.19
NHE1	1.20	0.82	0.151	0.15
NHE2	1.31	0.87	0.285	0.33
NHE3	1.38	0.50	0.083	<0.01
HMGCS2	1.95	1.69	0.433	0.70
ATP1	1.73	1.80	0.273	0.87

fewer protons are released and less acid is produced when hexose ferments to butyrate, as opposed to acetate or propionate (Owens and Goetsch, 1988).

In a companion study, Chen et al. (2012) reported decreased bacterial density in AR steers and differences in the diversity of bacterial communities for both epimural bacteria and bacteria from ruminal digesta. The lower bacterial density might indicate that AR produced fewer VFA compared with AS. The authors also found that a higher population of epimural bacteria is associated with higher ruminal pH and lower VFA production in AR steers, which indicates that epimural bacteria may play a stimulatory role in VFA absorption, and an increase in the number of bacteria from ruminal digesta is associated with a higher proportion of butyrate in AR steers. Further studies are necessary to determine whether and how microbial or host factors affect VFA production and molar proportion of butyrate in animals fed a common diet.

Gene Expression

Epithelial Intracellular pH Regulation. Absorption of VFA from the rumen occurs by simple diffusion of undissociated VFA into the ruminal epithelial cells, followed by subsequent dissociation in the cell, or facilitated diffusion of dissociated VFA by transport proteins located on the membrane of the epithelial cells (Connor et al., 2010). We hypothesized that a difference in expression of these transport proteins might be related to ruminal pH and could therefore play a role in resistance to rumen acidosis.

Graham et al. (2007) showed that MCT1 is located on the basal side of ruminal epithelial cells and is responsible for the removal of protons from the epithelial cell by cotransporting dissociated VFA, lactate, and ketones with H^+ into the blood (Kirat et al., 2006). Bilk et al. (2005) proposed that DRA and PAT1 are responsible for neutralizing acid in the rumen by exporting bicarbonate from epithelial cells and importing dissociated VFA. Penner at al. (2009a) observed a tendency for SARA-resistant sheep to have a greater capacity for bicarbonate-dependent uptake of acetate, which may have been due to increased expression of DRA, PAT1, or both. Therefore, we expected to see greater expression levels of MCT1, DRA, and PAT1 in AR; however, the levels were similar between AR and AS ($P > 0.10$; Table 6). Differences in VFA absorption could also have been caused by differences in ruminal motility, surface area of ruminal papillae, or blood flow rate, which would allow for greater diffusion of VFA from the rumen; however, these variables were not evaluated in the current study. Future research is necessary to determine the extent to which these factors affect resistance to SARA. It is also important to note the difficulty of obtaining representative ruminal papillae samples because of the size of the rumen. In this study, biopsies of papillae were taken from the same location of the rumen to compare AR and AS steers, but it is not known whether these samples were representative of the whole rumen, and future studies need to address this concern.

The NHE proteins are another mechanism by which the ruminal epithelial cells maintain intracellular pH. Graham et al. (2007) showed that NHE1, NHE2, NHE3, and NHE8 are present in ruminal epithelial cells. The NHE1 and NHE3 are located on the apical side of the epithelial cell; they import Na^+ to the cell and export H^+ to the rumen, whereas NHE2 imports Na^+ to the cell but exports H^+ to the extracellular space (Connor et al., 2010). We expected to find lower expression levels of NHE1 and NHE3 in AR, because they would result in protons returning to the ruminal lumen and greater expression of NHE2 because this would result in removal of a proton from the system. We found that expression levels of NHE1, NHE2, and NHE3 were consistently higher in AR compared with AS steers, but only NHE3 was significantly different ($P < 0.01$). Although a greater abundance of mRNA does not necessarily mean greater protein production or its activity unless transcription limits protein synthesis, long-term regulation of NHE3 is achieved

through changes in transcription of the gene (Zachos et al., 2005), and mRNA abundance of this gene likely reflects the protein synthesis.

Graham et al. (2007) proposed that apically located NHE proteins reduce extracellular pH and promote uptake of undissociated VFA by epithelial cells. Greater uptake of undissociated VFA via simple diffusion into ruminal epithelial cells of AR might ultimately result in increasing expression of NHE3 to prevent reductions in intracellular pH caused by release of protons from dissociation of VFA inside the cell.

Ruminal pH can differ according to the location where it is measured. Although we observed higher pH in the ruminal digesta for AR, as compared with AS, it is possible that pH near the epithelial cells might have been lower because of increased NHE3 expression. This would promote the formation of undissociated VFA near the epithelia and successive diffusion into ruminal epithelial cells. The VFA would subsequently dissociate in the epithelial cell, which might contribute to increased NHE3 expression to avoid the reduction in intracellular pH caused by excess proton accumulation, by exporting protons back into the rumen. Further studies are necessary to determine whether increased NHE3 expression is a consequence or a cause of high ruminal pH.

Krishnan et al. (1999) found that the presence of VFA stimulates absorption of sodium in the colon of rats and that VFA with longer carbon chain lengths have greater effects on sodium absorption, which was demonstrated again by Kiela et al. (2001). Greater sodium absorption may be due to NHE proteins that exchange sodium and protons to regulate intracellular pH, and these findings are consistent with the greater NHE3 expression that we observed for the AR steers. Greater molar proportion of butyrate in AR might have contributed to higher expression of NHE3. Further, butyrate is high in proliferative effects in ruminal epithelial cells (Sakata and Tamate, 1978), which may have increased the surface area of ruminal papillae and subsequently increased absorption of VFA from the rumen. However, contrary to these data, Laarman et al. (2012) observed that calves fed milk replacer and calf starter increased molar proportion of butyrate but decreased expression of NHE3 compared with calves fed milk replacer only. In contrast, expression of MCT1 was greater for calves fed milk replacer and starter in the study by Laarman et al. (2012), which indicates that the ruminal epithelial cells of young ruminants may have relied on MCT1 to a greater extent than on NHE3 to remove protons from the cells. However, the exact mechanism is not known and warrants further investigation.

Epithelial Cell Metabolism. Albrecht et al. (2008) demonstrated the importance of the Na^+/K^+ ATPase to the function of NHE proteins. Because AR animals had greater expression of NHE3 compared with AS animals, we hypothesized that expression of the Na^+/K^+ ATPase must be greater in AR animals to remove Na^+ from the epithelial cells. However, the current study found no difference in expression of this gene ($P > 0.10$).

Butyrate is extensively metabolized to BHBA by ruminal epithelial cells (Sehested et al., 1999). Lane et al. (2002) showed that 3-hydroxy-3-methylglutaryl-CoA synthase (**HMGCS**) is the rate-limiting enzyme in hepatic ketogenesis and proposed that this holds true for ruminal epithelial cells. Penner et al. (2009a) observed greater plasma BHBA concentration in SARA-resistant sheep, which indicates that expression or activity of HMGCS2 in the ruminal epithelial cells might be greater for SARA-resistant sheep, although it was not measured. As such, we expected that a higher proportion of HMGCS2 would also be expressed in AR steers. However, we did not observe a difference in HMGCS2 expression ($P > 0.10$) between AR and AS animals. In agreement with our findings, Lane et al. (2002) showed that the presence of VFA in the rumen does not influence the expression of genes that regulate ketogenesis in growing lambs.

Plasma Metabolites and Hormones

Plasma glucose, insulin, BHBA, and NEFA concentrations were not different between AR and AS steers ($P > 0.10$; Table 7). We expected plasma BHBA to be higher in AR steers because of an increased rate of ketogenesis, which Penner et al. (2009a) found in their

Table 7. Comparison of plasma blood metabolite and hormone concentration between acidosis-resistant and acidosis-susceptible steers

Variable	Resistant	Susceptible	SE	*P*-value
Glucose, mg/dL	81.1	76.2	4.20	0.45
BHBA, mg/dL	22.1	15.9	2.32	0.14
NEFA, mEq/L	114	77	18.5	0.24
Insulin, μIU/dL	90.2	64.2	24.38	0.49

study. Although concentrations of butyrate and BHBA are not necessarily the same as their production, it is probable that greater absorption of butyrate saturates ketogenesis pathways because BHBA does not increase proportionally with absorption of butyrate (Krehbiel et al., 1992, Rémond et al., 1993), which may explain why no difference was observed between plasma BHBA concentrations in AR and AS steers.

Implications

The current study reports several interesting preliminary findings about the differences between AR and AS steers. However, the results need to be interpreted with caution. Because we needed to evaluate a few extreme animals in ruminal pH responses to a high-grain diet for the current study, we had only 3 AR and 3 AS steers, which may not have provided sufficient statistical power to detect significant differences in some response variables. In addition, although we tried to avoid confounding effects of dietary factors by feeding a common diet at a predetermined intake level, rates of VFA production and VFA absorption were not measured for this study. As such, the specific causes for lower VFA concentration in AR steers compared with AS steers could not be identified. Further research is warranted to confirm our preliminary findings and identify specific mechanisms affecting the extent of resistance to SARA.

CONCLUSIONS

The AR steers had lower total VFA concentration, higher molar proportion of butyrate in the ruminal fluid, and greater NHE3 expression in ruminal epithelial cells compared with AS steers. These findings suggest that higher ruminal pH in AR might be partly due to increased VFA absorption via simple diffusion or lower VFA production. Further research on ruminal morphology and VFA absorption and subsequent responses in ruminal epithelial cells are needed to improve the understanding of why SARA occurs in some animals but not in others fed a common diet.

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