An energy-rich diet enhances expression of Na⁺/H⁺ exchanger isoform 1 and 3 messenger RNA in rumen epithelium of goat

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ABSTRACT: Rumen epithelial Na⁺/H⁺ exchanger (NHE) catalyzes the exchange of extracellular Na⁺ for intracellular H⁺. Thus, it is of importance in the maintenance of Na and pH homeostasis of rumen epithelial cells. We have tested the hypothesis that an increase in energy and protein intake induces alterations of NHE isoform 1, 2, and 3 (NHE1, NHE1, and NHE3, respectively) mRNA abundance in the rumen epithelium of goats. Goats (n = 26) were randomly allocated to 2 experiments (n = 16 in Exp. 1, and n = 10 in Exp. 2) and fed either peanut straw ad libitum [PNS, n = 8 in Exp. 1, and n = 5 in Exp. 2; 600 kJ of ME/(kg⁰.⁷⁵ ∙ d)] or PNS + concentrate [CF, n = 8 in Exp. 1, and n = 5 in Exp. 2; 1,000 kJ of ME/(kg⁰.⁷⁵ ∙ d)] for 42 d. Concentrate (400 g/d) was given daily (0800 to 1700 h) in 4 equal portions at 3-h intervals. In Exp. 1, the goats were euthanized 2 h after the last portion of concentrate was fed, and in Exp. 2, the goats were euthanized after a fasting period of 16 h. In Exp. 1, goats in the CF treatment exhibited a greater ruminal short-chain fatty acid (SCFA) concentration (140.6 ± 1.30 mM) compared with those in the PNS treatment (114.3 ± 3.11 mM; P < 0.001), and pH decreased from 6.9 ± 0.09 to 5.9 ± 0.04 (P < 0.001). Correspondingly, the mRNA expression of NHE1 and NHE3 in the rumen epithelium was greater by 20% (P = 0.041) and 25% (P = 0.043) for goats in the CF treatment than for those in the PNS treatment. However, in Exp. 2, 16 h of fasting abolished differences in ruminal SCFA concentration, pH, and NHE mRNA expression between goats in the CF and PNS treatments. In both Exp. 1 and 2, a positive correlation was observed between ruminal SCFA concentration and expression of mRNA in NHE1 and NHE3, whereas expression was negatively correlated with ruminal pH. In in vitro studies with isolated rumen epithelial cells from goats fed dried grass, exposure to pH of 6.8 or to 20 mM SCFA increased (P < 0.01) NHE1 and NHE3 mRNA expression, as compared with exposure to pH of 7.4 or the absence of SCFA. A combination of reduced pH (6.8) and SCFA (20 mM) further enhanced (P < 0.05) NHE1 and NHE3 mRNA expression, indicating synergy between an increased concentration of SCFA and low pH (P < 0.05). Messenger RNA expression of NHE2 did not vary in vitro with pH (6.8) or SCFA (20 mM) or in vivo in Exp. 1 and 2. Thus, diet-dependent rumen epithelial NHE1 and NHE3 expression is probably related to ruminal SCFA concentration and pH, but that is not the case with NHE2.

Key words: diet, goat, pH, rumen epithelial Na⁺/H⁺ exchanger expression, rumen fermentation, short-chain fatty acid

INTRODUCTION

The functions and morphology of the rumen epithelium adapt to increased carbohydrate feeding through enlargement of papillae and enhanced absorptive capacity (Dirksen et al., 1984). The underlying mechanisms of this adaptation are not completely clear, but luminal factors, such as short-chain fatty acids (SCFA), most probably contribute to the physiological alterations of the rumen epithelium (Sakata, 2004; Shen et al., 2004; Etschmann et al., 2009). The Na⁺/H⁺ exchanger (NHE) isoforms 1, 2, and 3 (NHE1, NHE2, and NHE3, respectively) are associated with the bovine (Graham et al., 2007) and ovine (Schweigel et al., 2005) rumen epithelium. The NHE activity of the rumen epithelium is closely linked to the presence of SCFA (Gäbel et al., 1991b). In the rat colon, SCFA modulate NHE3 gene expression (Musch et al., 2001). However, previous feeding trials with dairy cows (Penner et al., 2009) and sheep (Priessnitz, 2007) have reported that the feeding regimen does not affect the expression of mRNA coding for NHE proteins in the rumen epithelium. In the

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study by Penner et al. (2009), epithelial samples were collected after cow transportation, whereas Priessnitz (2007) collected samples from sheep 15 h after the last feeding. In these experiments, rumen fermentation was likely complete, and the effect of diet-induced differences in SCFA and pH on NHE1 to NHE3 transcription may have been minimal. Rumen fermentation varies not only with the composition of the diet, but also with the time after feeding (Bergman, 1990). Therefore, the feeding regimes (Shabi et al., 1999) and the composition of the diet influence the actual ruminal SCFA concentration and pH (Swanson et al., 2000).

We hypothesized that the dietary effects on rumen epithelial mRNA expression of NHE are associated with rumen fermentation. The current experiments therefore studied in vivo the effects of diet [peanut straw (PNS) vs. peanut straw supplemented by concentrate (CF)] and in vitro the effects of SCFA and pH on NHE1, NHE2, and NHE3 mRNA expression in rumen epithelial cells.

**MATERIALS AND METHODS**

The experimental design and procedures were approved by the Animal Care and Use Committee of Nanjing Agricultural University following the requirements of the Regulations for the Administration of Affairs Concerning Experimental Animals (The State Science and Technology Commission, 1988).

**Experimental Design and Goat Management**

Twenty-six goats (Boer × Yangtze River Delta White), aged 2 mo when the experiment commenced, were used for the feeding trial and 9 adult goats fed dried grass were used for cell culture. Before the trial, the goats consumed peanut straw ad libitum with progressively increasing amounts of concentrates for 30 d to adapt to concentrate feeding of 400 g/d. This adaptation period was followed by a feeding trial of 42 d. The goats (13.4 ± 0.8 kg of BW) were randomly allocated to 2 experiments (n = 16 in Exp. 1, and n = 10 in Exp. 2) and received a diet of either PNS or CF. In the feeding trial, the goats in both Exp. 1 (n = 8 for each treatment) and Exp. 2 (n = 5 for each treatment) were fed according to a normal continuous-feeding protocol, with concentrate (400 g) being provided in 4 equal portions at 0800, 1100, 1400, and 1700 h daily to goats in the CF treatment. Peanut straw was given once daily at 0800 h to both the CF and PNS groups. Water was freely available to all goats during the experiment. On d 43, the goats in Exp. 1 were fed following the normal continuous-feeding protocol and were slaughtered at 1900 h. In Exp. 2, the goats in both groups were fasted for 16 h before slaughter. All goats were slaughtered at a local slaughterhouse, with 4 goats (2 per treatment) being slaughtered each day.

The intakes of CF and of PNS were recorded daily, and the BW was determined weekly. The supplement of PNS was adjusted according to BW gain to ensure approximately 30% orts. Feed was sampled at wk 1 and 6 for chemical analysis (Table 1).

**Sample Collection**

Immediately after slaughter, rumen fluid was collected and strained through 3 layers of cheesecloth, transferred into plastic bottles containing mercuric chloride, and stored at −20°C until analysis. Rumen tissue from the ventral blind sac (2 × 2 cm) was quickly excised, and loosely adherent feed particles were removed, placed in ice-cold PBS (pH 7.4), and repeatedly rinsed until the PBS remained clear. The muscle layers were removed, and the epithelium was transferred into liquid N within 5 min and stored at −80°C until analyzed for NHE1, NHE2, and NHE3 mRNA abundance.

**Cell Culture**

The rumen epithelia from 9 adult goats (Boer × Yangtze River Delta White) fed dried grass were collected for cell culture. The epithelium from the rumen atrium ruminis of each goat was prepared as described

### Table 1. Dry matter intake and chemical composition of diets<sup>1,2</sup>

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentrate&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Peanut straw&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>As the feeding trial commenced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI of concentrate, g/d</td>
<td>356.2 ± 14.9</td>
<td>—</td>
</tr>
<tr>
<td>DMI of peanut straw, g/d</td>
<td>528.5 ± 22.0</td>
<td>614.3 ± 30.7</td>
</tr>
<tr>
<td>Total DMI, g/d</td>
<td>884.7 ± 36.9</td>
<td>614.3 ± 30.7</td>
</tr>
<tr>
<td>BW of the goats, kg</td>
<td>13.7 ± 1.0</td>
<td>13.1 ± 1.2</td>
</tr>
<tr>
<td>ME, kJ/(kg&lt;sup&gt;0.75&lt;/sup&gt;·d)</td>
<td>1,013.8 ± 42.3</td>
<td>593.7 ± 29.7</td>
</tr>
<tr>
<td>On slaughter day&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI of concentrate, g/d</td>
<td>356.3 ± 15.9</td>
<td>—</td>
</tr>
<tr>
<td>DMI of peanut straw, g/d</td>
<td>802.1 ± 35.9</td>
<td>645.9 ± 37.9</td>
</tr>
<tr>
<td>Total DMI, g/d</td>
<td>1,158.4 ± 51.9</td>
<td>645.9 ± 37.9</td>
</tr>
<tr>
<td>BW of the goats, kg</td>
<td>18.6 ± 1.0</td>
<td>14.7 ± 1.2</td>
</tr>
<tr>
<td>ME, kJ/(kg&lt;sup&gt;0.75&lt;/sup&gt;·d)</td>
<td>1,059.1 ± 47.4</td>
<td>601.8 ± 35.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>The values for DMI, ME, and BW are means ± SE. The energy and N levels in the feeding trial were 2.0× and 3.2× maintenance in the peanut straw + concentrate (CF) group and 1.2× and 1.3× maintenance in the peanut straw (PNS) group, respectively.

<sup>2</sup>PNS: n = 8 in Exp. 1 and n = 5 in Exp. 2; CF: n = 8 in Exp. 1 and n = 5 in Exp. 2.

<sup>3</sup>DML and ME in Exp. 1 (n = 16) with consecutive feeding; in Exp. 2 (n = 10), the goats were fasted for 16 h before slaughter. The analysis was performed in duplicate.

<sup>4</sup>The concentrate was composed of ground corn, soybean meal, cottonseed bran, wheat bran, calcium phosphate, limestone, trace mineral salt, and vitamin premix (vitamins A, D, and E).
above and digested with 0.25% trypsin to obtain individual epithelial cells. Viability of the cells was confirmed by staining with trypan blue, and cell density was adjusted to \( \times 1,000,000 \) cells/mL in high-glucose (4,500 mg/L) Dulbecco’s modified Eagle’s medium (Gibco BRL, Grand Island, NY) with 10% fetal bovine serum (Sijiqing, Hangzhou, China), 2 mM l-glutamine (Sunshine, Nanjing, China), 100 U/mL of penicillin, and 100 mg/mL of streptomycin. The cells were seeded into 25-cm\(^2\) plastic tissue-culture flasks (Corning, Corning, NY) and incubated at 37°C in an incubator.

Reverse-Transcription PCR

RNA Isolation and Semiquantitative

pH and SCFA Determination

The pH of rumen fluid was measured with a Mettler Toledo Delta 320 pH meter (Mettler-Toledo Group, Halstead, UK), and SCFA were measured using an Agilent HP6890N gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a flame-ionization detector and a 30 m × 0.32 mm i.d. × 0.25 μm film thickness HP-FFAP capillary column (Hewlett-Packard, Palo Alto, CA). Nitrogen was used as the carrier gas (99.99% purity), with a constant flow rate of 2.8 mL/min and a split ratio of 1:30. The temperature of the capillary column was set to 140°C for 4 min and then increased at 25°C/min to 240°C. The temperatures of the injection port and the flame-ionization detector were set to 180 and 250°C, respectively. Crotonic acid was used as an internal standard.

RNA Isolation and Semiquantitative Reverse-Transcription PCR

Total RNA was isolated from rumen epithelium samples and from cultured cells with acid guanidinium thiocyanate-phenol-chloroform (Chomczynsky and Sacchi, 2006). The RNA concentration was then quantified by measuring absorbance at 260 nm using a BioPhotometer (Eppendorf, Hamburg, Germany). The absorption ratio (260/280 nm) of all samples was between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis through a 1.4% agarose-formaldehyde gel to verify integrity. Concentration of RNA was adjusted to 1 \( \mu \)g/\( \mu \)L based on optical density, and the RNA was stored at −80°C.

The primers for NHE1, NHE2, and NHE3 were used as described by Graham et al. (2007). The primer for 18S rRNA was designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). All primers were carefully selected and synthesized by Invitrogen Life Technologies (Shanghai, China).

Total RNA (2 μg) was reverse-transcribed by incubation at 37°C for 1 h in a 25-μL mixture consisting of 100 U of Moloney murine leukemia virus reverse transcriptase (Fermentas, Burlington, Ontario, Canada), 8 U of ribonuclease inhibitor (Biouniquer, Hong Kong, China), 21 mM random hexamer primers (Invitrogen, Shanghai, China), 50 mM Tris-HCl pH 8.3, 3 mM MgCl\(_2\), 75 mM KCl, 10 mM dithiothreitol, and 0.8 mM each deoxynucleotide 5′-triphosphate (MBio, Shanghai, China). The reaction was terminated by heating at 95°C for 5 min, followed by immediate placement on ice. Of the reverse-transcription reaction mix, 2 μL was used for PCR in a final volume of 25 μL containing 2.5 μL of 10× Taq buffer with KCl (Fermentas), 2 μL of a mixture of 2.5 mM deoxynucleotide 5′-triphosphate, 1 U of Taq DNA polymerase (Fermentas), and 10 pmol of each forward and reverse primer. The conditions of PCR amplification were as follows: 1 cycle at 94°C for 5 min, 15 to 30 cycles at 94°C for 30 s, 58°C for 35 s, and 72°C for 40 s, with a final extension cycle at 72°C for 10 min. The PCR products from each reaction were sent to Haojia Biotech. Ltd. (Nanjing, China) for sequencing to verify specificity. The PCR-amplified fragments were run beside molecular weight markers on 2% agarose gels stained with ethidium bromide. Gels were photographed with a digital camera, and the net intensities of individual bands were measured using Kodak Digital Science 1D software (Eastman Kodak Company, Rochester, NY). The semiquantitative measurement of gene expression was normalized to 18S rRNA. If not otherwise stated, experiments were performed in triplicate.

Statistical Analyses

All data were expressed as means ± SE. Differences were considered significant when \( P < 0.05 \), as tested by the independent samples \( t \)-test and 1-way ANOVA. Correlations were calculated as Pearson correlation coefficients, using data from both Exp. 1 and 2. The GLM with relevant interactions was used to determine the significance of differences in mRNA abundance in vitro. All statistical analyses were performed by using SPSS (SPSS Inc., Chicago, IL) software packages.

RESULTS

Rumen Epithelial mRNA Expression, Rumen SCFA, and pH

In Exp. 1 (Table 2), total SCFA concentration was 23% greater (\( P < 0.001 \)) in the CF group and pH was almost 1 U less (\( P < 0.001 \)) than in the PNS group. Messenger RNA expression of NHE1 and NHE3 in the CF group (Figure 1a) was increased by 20% (\( P = 0.041 \)) and 25% (\( P = 0.043 \)), respectively, as compared...
with mRNA expression in the PNS group. However, the expression of NHE2 mRNA did not differ between treatments.

The experimental design of Exp. 2 was similar to that of Exp. 1, with the exception that the goats were slaughtered 16 h after the last feeding. The total SCFA concentration and pH did not differ \((P = 0.94, P = 0.93)\) between the CF and PNS groups as a result of overnight fasting (Table 2). The expression of mRNA from NHE1, NHE2, and NHE3 also did not differ \((P = 0.64, 0.44, 0.43)\) between the CF and PNS groups (Figure 1b).

### Correlations Between Ruminal SCFA, pH, and NHE mRNA Expression

Total rumen SCFA concentrations (Table 3) were positively correlated with mRNA expression of NHE1 \((r = 0.44, P = 0.046)\) and NHE3 \((r = 0.46, P = 0.018)\). Conversely, ruminal pH (Table 3) was negatively corre-

<table>
<thead>
<tr>
<th>Item</th>
<th>Exp. 1 CF</th>
<th>Exp. 1 PNS</th>
<th>SEM</th>
<th>(P)-value</th>
<th>Exp. 2 CF</th>
<th>Exp. 2 PNS</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.9</td>
<td>6.9</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>6.9</td>
<td>6.9</td>
<td>0.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Total SCFA, mM</td>
<td>140.6</td>
<td>114.3</td>
<td>3.75</td>
<td>&lt;0.001</td>
<td>95.2</td>
<td>94.3</td>
<td>4.97</td>
<td>0.93</td>
</tr>
<tr>
<td>Acetate, mM</td>
<td>96.9</td>
<td>82.4</td>
<td>2.32</td>
<td>&lt;0.001</td>
<td>61.9</td>
<td>65.3</td>
<td>3.47</td>
<td>0.65</td>
</tr>
<tr>
<td>Propionate, mM</td>
<td>26.9</td>
<td>19.2</td>
<td>1.38</td>
<td>0.001</td>
<td>17.4</td>
<td>18.6</td>
<td>1.25</td>
<td>0.64</td>
</tr>
<tr>
<td>Butyrate, mM</td>
<td>16.6</td>
<td>12.7</td>
<td>0.71</td>
<td>0.002</td>
<td>15.8</td>
<td>10.3</td>
<td>1.14</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*The intakes of energy and N were 2.0× and 3.2× maintenance in the peanut straw + concentrate (CF) group and 1.2× and 1.3× maintenance in the peanut straw (PNS) group, respectively.

\(n = 16\) in Exp. 1 (consecutive feeding), and \(n = 10\) in Exp. 2 (fasting for 16 h). The analysis was performed in triplicate. Values are means ± SE. \(P < 0.05\) is considered significant.
-related with mRNA expression of NHE1 (r = −0.55, P = 0.021) and NHE3 (r = −0.54, P = 0.012). However, neither ruminal SCFA nor pH was correlated with NHE2 mRNA expression (Table 3).

**Effect of Acid and SCFA Loading on NHE mRNA Expression in Cultured Rumen Epithelial Cells**

A decrease of pH from 7.4 to 6.8 increased NHE1 and NHE3 mRNA expression by 19 and 23% after 12 h (P = 0.011, P = 0.048) of culture and by 39 and 37% after 24 h (P = 0.003, P = 0.005) of culture, respectively (Figure 2). No interaction was observed between pH and incubation time for mRNA expression of NHE1 (P = 0.13) and NHE3 (P = 0.38). Messenger RNA expression of NHE2 was not changed by treatment (Figure 2).

The presence of SCFA (20 mM, pH 7.4) in the medium (Figure 3) increased NHE1 and NHE3 mRNA expression by 23 and 21%, respectively, after 12 h of culture (P = 0.005, P = 0.039) and by 34 and 27%, respectively, after 24 h of culture (P = 0.003, P = 0.004) as compared with the control (no SCFA). No interaction between SCFA and incubation time was observed in mRNA expression of NHE1 (P = 0.47) or NHE3 (P = 0.61). The expression of NHE2 was not influenced by SCFA.

Addition of acid (pH 6.8) or SCFA (20 mM, pH 7.4) to the culture medium (Figure 4) enhanced mRNA expression of NHE1 by 14% (P = 0.008) and 11% (P = 0.032), respectively, and enhanced mRNA expression of NHE3 by 14% (P < 0.001) and 10% (P = 0.003), respectively, after 24 h of incubation as compared with the control (pH 7.4, no SCFA). Moreover, a combined application of both low pH and SCFA (pH 6.8 + SCFA 20 mM) resulted in increased mRNA expression of NHE1 and NHE3 by 33% (P < 0.001) and 30% (P < 0.001), respectively, indicating a synergistic response in expression of NHE1 (P = 0.047) and NHE3 (P = 0.042) to pH and SCFA.

**DISCUSSION**

In sheep, goats, and cows, the absorptive capacity of the rumen epithelium is closely associated with feed intake. Absorptive capacity increases with concentrate feeding (Dirksen et al., 1984; Shen et al., 2004; Etschmann et al., 2009) but decreases drastically after food deprivation (Gäbel et al., 1993). After introduction to a concentrate diet, changes occur in the size and number of papillae in the rumen over a period of 4 to 6 wk (Dirksen et al., 1984; Shen et al., 2004) or over 3 to 4 wk if the concentrate diet is introduced rapidly (Bammink et al., 2008). Functional adaptation also includes changes in ion transport mechanisms (Gäbel et al., 1993; Sehested et al., 2000; Uppal et al., 2003a,b), which occur much faster than morphological alterations. In a recent in vitro study, we observed enhanced NHE-mediated Na+ absorption in the rumen epithelium within 1 wk after changing sheep from a roughage-based to a concentrate-based diet. Therefore, enlargement of the rumen epithelial absorptive surface was preceded by an increase in NHE activity that might enhance Na transport (Etschmann et al. 2009).

However, previous feeding trials with dairy cows (Penner et al., 2009) and sheep (Priessnitz, 2007) have shown that the expression of mRNA in the rumen epithelium is not affected by feed composition and nutritional intake. Penner et al. (2009) reported that ruminal mRNA of proteins involved in SCFA absorption, ketogenesis, and pyruvate metabolism, as well as in Na+/H+ exchange, remained unchanged in dairy cows fed a low- or high-concentrate diet. Similarly the rumen epithelial mRNA of NHE, Na/K-adenosine triphosphatase, monocarboxylate-transporter 1, anion exchanger 2, and vacuolar-type H-adenosine triphosphatase did not differ between intake of hay or hay supplemented with concentrate in sheep (Priessnitz, 2007). In addition, the expression of mRNA of IGF-1 and IGF type 1 receptor in the rumen epithelium of goats was not affected by an energy-rich diet (Shen et al., 2004). The lack of effect of the feeding regimen on ruminal mRNA expression in these studies is surprising because diet-dependent changes in Na transport via NHE (Shen et al., 2004; Etschmann et al., 2009) or Cl transport via the anion exchanger (Gäbel et al., 1987) have been well documented. Furthermore, an increase in NHE-mediated Na transport is also closely related to NHE mRNA expression (Laghmani et al., 1997). One possible explanation is that alterations in intake as a result of transportation or fasting before slaughter may alter mRNA expression of NHE transporters at the time of tissue sampling. Nevertheless, the current work shows that NHE1 and NHE3 mRNA expression in the rumen epithelium is enhanced by a concentrate as compared with a forage diet.

### Table 3. Correlation between ruminal pH, total short-chain fatty acid (SCFA), and expression of Na+/H+ exchanger (NHE) isoform 1, 2, and 3 mRNA

<table>
<thead>
<tr>
<th>Item</th>
<th>NHE1</th>
<th>NHE2</th>
<th>NHE3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
<td>r</td>
</tr>
<tr>
<td>pH</td>
<td>−0.55</td>
<td>0.021</td>
<td>−0.25</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>0.44</td>
<td>0.046</td>
<td>0.32</td>
</tr>
</tbody>
</table>

1Correlation coefficients are calculated as Pearson correlation coefficients. The data were collected from Exp. 1 and 2 (n = 26), and analyses were performed in duplicate. P < 0.05 was considered significant.
The adaptation of the rumen epithelium to a change in diet is most probably influenced either by systemic factors, such as IGF-1 (Shen et al., 2004) and insulin, or by local and luminal factors, such as rumen-generated SCFA and pH (Gäbel et al., 1991a; Uppal et al., 2003a,b). The effects of hormones cannot be excluded in the current experiment, but we should emphasize that both SCFA and pH are considered potential triggers of morphological and functional adaptation of the rumen epithelium to a change of diet (Dijkstra et al., 1993; Shen et al., 2005; Bannink et al., 2008) and modulators of gene expression in the intestine (Musch et al., 2001). Rumen fermentation varies not only with the composition of the diet, but also with the time after feeding (Bergman, 1990). Therefore, the feeding regimen (Shabi et al., 1999), in addition to the feed composition and nutritional intake, influences the actual status of ruminal SCFA concentration and, in turn, ruminal pH (Swanson et al., 2000).

Ruminal total SCFA concentration was 23% greater and pH was 1 U less in goats in the CF compared with the PNS group when measured 2 h after the last feeding. Shabi et al. (1999) observed a reduced diurnal variation in ruminal pH in dairy cows fed 4 times per day as compared with those fed twice per day. Providing a fixed ration of hay and high-cereal concentrates to lactating cows 24 times per day, as compared with twice daily, reduced the diurnal variation in ruminal SCFA concentrations and pH (Sutton et al., 1986). Furthermore, in our previous feeding experiment with goats,
in which the feeding regimen was identical to that of the current feeding trial, the rumen contents were collected postprandially through a rumen fistula every 2 h for pH measurement. The data obtained confirmed that ruminal pH was significantly less when CF diets, as compared with PNS diets, had been fed (W. Yang and Z. Shen, unpublished data). Hence, the fermentation pattern differed between the 2 groups, in accordance with our working hypothesis, and differed from that in the experiment by Priessnitz (2007), in which sheep were fed concentrate twice daily for 6 wk, with the last portion of concentrate being fed 15 h before the sheep were killed in the morning. Numerous studies have documented that fasted ruminants have much smaller rumen SCFA concentrations (Brown and Shaw, 1957). Early studies have revealed that gene expression can be modulated through changes in mRNA stability (Brawerman, 1987; Wilusz et al., 2001). Each individual mRNA has its intrinsic stability under a given condition (Chen et al., 2008). In opossum kidney cells, the NHE3 mRNA has a half-life of 8 h (Baum et al., 1996), whereas in mouse NIH-3T3 (embryonic fibroblast cell line) cells, the c-fos proto-oncogene transcript has a half-life of 10 to 15 min (Treisman, 1985; Shyu et al., 1989). The stability of an individual mRNA may change in response to a variety of extracellular stimuli. Any harmful agents, such as oxidants, stresses, and extreme temperatures, affect mRNA stability (Fan et al., 2002). Zhang et al. (1998) observed that fasting attenuates hepatic mRNA generation and accelerates its

Figure 3. In vitro effects of short-chain fatty acids (SCFA; 20 mM) on Na+/H+ exchanger 1, 2, and 3 (NHE1, NHE2, and NHE3, respectively) mRNA expression in rumen epithelial cells of goats fed dried grass after cells were incubated for 12 and 24 h. A) Representative electrophoresis images of reverse-transcription (RT) PCR products for NHE1 to NHE3 mRNA (above) and co-amplified 18S rRNA (below). L = DNA ladder. The primers for NHE1 to NH3 were used as described by Graham et al. (2007). B) Statistical analysis of NHE1 to NHE3 mRNA expression. The 20 mM SCFA mixture contained (mM) 12 sodium acetate, 5 sodium propionate, and 3 sodium butyrate. Messenger RNA expression was quantified with RT-PCR and expressed as arbitrary units relative to 18S rRNA. The rumen epithelium was collected from 8 goats (n = 8), and the experiment was performed in triplicate. Values are means ± SE. Values within a group without a common letter (a, b) are significantly different (P < 0.05). The interactions of SCFA and time are depicted.
rate of degradation in cytoplasm, and it may also have limited differences from NHE mRNA expression in the studies of Priessnitz (2007) and Penner et al. (2009).

In Exp. 2, rumen concentration of SCFA, ruminal pH, and concentration of NHE mRNA in the rumen epithelium did not differ between the 2 groups under fasted conditions. Consequently, fasting may have attenuated the possible stimulating effects of high energy intake in the CF group during the previous 4-meal concentrate feeding. A positive correlation was observed between ruminal mRNA expression of NHE1 and NHE3 and SCFA concentration, whereas this relationship was negative for pH. These data are in general agreement with those of Musch et al. (2001), who stimulated colonic fermentation by the ad libitum feeding of pectin to rats and found an increase in mRNA and protein expression of NHE3, but not NHE2. The mechanisms of differential regulation of NHE isoforms have remained unclear. However, previous studies in rat ileal and colonic epithelial cells (Lucioni et al., 2002), rabbit ileal brush-border cells (Wormmeester et al., 1998), and human intestinal Caco-2 cells (Hecht et al., 2004) have indicated that the regulation of NHE might be tissue and isoform specific.

In the present in vitro experiments, mRNA expression of NHE1 and NHE3 was stimulated by reduced pH or SCFA after both 12 and 24 h of cell culture. This was consistent with previous reports that reduced pH

**Figure 4.** In vitro effects of acid (pH 6.8), short-chain fatty acids (SCFA; 20 mM), or acid (pH 6.8) + short-chain fatty acids (SCFA; 20 mM) on Na+/H+ exchanger 1, 2, and 3 (NHE1, NHE2, and NHE3, respectively) mRNA expression in rumen epithelial cells of goats fed dried grass after cells were incubated for 24 h. A) Representative electrophoresis images of reverse-transcription (RT) PCR products for NHE1 to NHE3 mRNA (above) and co-amplified 18S rRNA (below). L = DNA ladder. The primers for NHE1 to NHE3 were used as described by Graham et al. (2007). B) Statistical analysis of NHE1 to NHE3 mRNA expression. In groups with pH 6.8 treatment, the medium was acidified by 1 N HCl. The 20 mM SCFA contained (mM) 12 sodium acetate, 5 sodium propionate, and 3 sodium butyrate. The mRNA expression was quantified with RT-PCR and expressed as arbitrary units relative to 18S rRNA. The rumen epithelium was collected from 9 goats (n = 9) and the experiment was performed in triplicate. Values are means ± SE. Values within a group without a common letter (a–c) are significantly different (P < 0.05). The interactions of pH and SCFA are depicted.
stimulates NHE1 and NHE3 expression in certain types of cells (opossum kidney clone P cells, renal epithelial cells, and fibroblasts) after 6 or 24 h treatment (Moe et al., 1995; Yang et al., 2000) and further shows that SCFA and pH act as independent factors, but when combined, they synergistically induce mRNA expression. These results suggest that the enhanced NHE1 and NHE3 mRNA expression in Exp. 1 was a result of both the increased SCFA concentration and reduced pH. This correlation between fermentation pattern and NHE expression is of significant physiological importance because low pH in combination with greater SCFA concentrations enhances uptake of SCFA and hence of protons, which must be excreted from the epithelial cells to maintain a constant intracellular pH (pHi). It has been shown in studies with isolated cells from the ovine rumen that both isoforms (NHE3 and NHE1) are involved in pH regulation (Schweigel et al., 2005). Recent studies in our laboratory (Rabbani et al., 2011) have clearly shown that NHE3 is located in the apical membrane, and its activity can be considered as a first line of defense to prevent reducing the pH during uptake of protonated SCFA by the rumen epithelium. It is important to emphasize that NHE activity in the apical membrane is coupled with Cl− uptake and HCO3− secretion (Gäbel et al., 1991a), which buffers protons in the rumen and contributes to the increase in ruminal pH resulting from SCFA absorption.

This study is the first to reveal correlations among dietary energy intake, intraruminal SCFA concentration, pH, and abundances of NHE1 and NHE3 mRNA. It is in agreement with our previous observation (Shen et al., 2004) that an energy-rich diet enhances NHE activity in the rumen epithelium. It is also consistent with the observations of Laghmani et al. (1997), who reported that in vivo metabolic acidosis increases the amount of mRNA transcript for NHE3, which corresponds to quantitatively similar increases in NHE3 protein abundance and transport activity in thick ascending limb cells isolated from the rat kidney. Musch et al. (2001) also demonstrated in rats that luminal SCFA modulate colonic Na absorption by stimulating protein, mRNA, and brush-border activity of NHE3.

At the present time, the way in which the presence of SCFA or a low pH is sensed by ruminal epithelial cells and finally transformed into increased mRNA expression is unclear. However, we should mention that rumen motility is severely reduced during rumen acidosis (Crichlow, 1989). Convincing evidence has been presented that the concentration of undissociated SCFA causes the contractions of the reticulo-rumen to cease (Gregory, 1987). The inhibition is assumed to be mediated by the excitation of acid-sensitive receptors that lie in the rumen wall (Crichlow and Leek, 1986) and that are located at the basement membrane of the forestomach epithelium (Harding and Leek, 1972, 1973). In addition to the acid-sensitive receptors in the rumen wall, a proton-sensor site is commonly accepted as being located at the cytosolic carboxy-terminal of NHE to modulate the NHE response to acidification (Wakabayashi et al., 1992; Lacroix et al., 2004). The regulation of NHE activity in the digestive tract is achieved by altering the expression of NHE mRNA and protein (Yun et al., 1993; Doble et al., 2002; Hayashi et al., 2002; Lucioni et al., 2002; Kiela et al., 2007) and by posttranslational mechanisms (He and Yun, 2010). Interactions between these receptors and the transport properties of the rumen epithelium are not known at the present time, but the regulation of pHi in isolated rumen epithelial cells is mediated by NHE1 and NHE3, as has been shown by Schweigel et al. (2005) and Etschmann et al. (2006) with specific NHE inhibitors.

Further studies that attempt to estimate gene transcription of the rumen epithelium should focus attention on the ruminal pH and SCFA concentration by taking samples at maximal rumen fermentation. Furthermore, the expression of NHE protein in the rumen epithelium should be included because the real actor, which catalyzes the exchange of extracellular Na+ for intracellular H+, is NHE protein. In conclusion, the current study reveals that the phenotypic responses of ruminal SCFA and pH to dietary intake are accompanied by modified expression of the mRNA of NHE1 and NHE3. These data are in contrast to previous studies that have a similar experimental design; these differences can most probably be explained on the basis of the fermentation pattern and time of tissue sampling.

**LITERATURE CITED**


Na⁺/H⁺ exchanger isoform 1 and 3 messenger RNA in goat rumen epithelium


