J. Dairy Sci. 97:5668–5675
http://dx.doi.org/10.3168/jds.2013-7807
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Dietary modulation of the expression of genes involved in short-chain fatty acid absorption in the rumen epithelium is related to short-chain fatty acid concentration and pH in the rumen of goats

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ABSTRACT

We have tested the hypothesis that increased concentrate intake induces mRNA abundance alterations of genes involved in short-chain fatty acid (SCFA) absorption in the rumen epithelium of goats and these changes of mRNA abundance are probably related to ruminal SCFA concentration and ruminal pH. Goats (n = 12) were randomly allocated to 2 groups and fed either a low-concentrate (LC) diet (10% concentrate; n = 6) or a medium-concentrate (MC) diet (35% concentrate; n = 6) in 2 equal portions daily. Goats were fed separately with their respective diet for 3 wk. The goats were slaughtered 6 h after the morning feeding on d 22. In vivo, goats in the MC treatment exhibited a greater ruminal SCFA concentration (81.9 m*M*) compared with those in the LC treatment (58.0 m*M*), and the pH decreased from 7.03 to 6.63. Correspondingly, mRNA expression of candidates for $SCFA^-$/HCO_3^- exchangers, namely downregulated in adenoma (*DRA*), putative anion transporter 1 (*PAT1*), and anion exchanger 2 (*AE2*) were increased in the MC group. Further, upregulation in monocarboxylate transporter 1 (*MCT1*) and monocarboxylate transporter 4 (*MCT4*) mRNA abundances was observed in the MC group. The expression of genes that help the rumen epithelial cells to maintain intracellular pH, including Na^+/H^+ exchanger 1 (*NHE1*) , *NHE2*, *NHE3*, vacuolar H^+ ATPase subunit B (*vH^+ ATPase*), and Na^+/K^+ ATPase pump subunit α1 (*Na^+/K^+ ATPase*) were also enhanced in the MC group relative to the LC group. During in vitro studies with isolated rumen epithelial cells from goats, exposure to a pH of 6.8 increased *MCT1* mRNA expression after 24 h of culture, whereas the mRNA expression of *AE2* was downregulated. The presence of SCFA (20 m*M*) in the medium increased *DRA*, *PAT1*, *AE2*, *MCT1*, and *Na^+/K^+ ATPase* mRNA expression. The expression of *vH^+ ATPase* was decreased by increased SCFA concentration. The mRNA expression of *MCT4* did not vary in vitro with pH (6.8) or SCFA (20 m*M*). The expression of *DRA* was increased by synergistic effects of higher SCFA concentration and lower pH, similar to that of *MCT1*. Thus, diet-dependent rumen epithelial mRNA expression changes in genes involved in SCFA absorption are probably related to ruminal SCFA concentration and pH.

Key words: rumen epithelium, gene expression, short-chain fatty acid absorption, short-chain fatty acid and pH

INTRODUCTION

Ruminal short-chain FA (**SCFA**) can be efficiently absorbed by the rumen epithelium via diffusion or a protein-mediated pathway (Leonhard-Marek et al., 2010; Aschenbach et al., 2011). Past research has demonstrated that feeding a high-concentrate diet could lead to enhanced SCFA absorptive capacity of rumen epithelium (Gäbel et al., 1991), even when no change occurred in the absorptive surface area of the rumen papillae (Sehested et al., 2000). Some experiments show that one reason for the changes of SCFA absorptive capacity is that diets could modulate the expression of genes encoding for proteins involved in SCFA absorption (Connor et al., 2010; Kuzinski and Röntgen, 2011).

The luminal factors, especially SCFA and pH, have been proven to contribute to the alterations in Na^+/H^+ exchanger 1 (***NHE1***) and *NHE3* expression in the rumen epithelium induced by diets (Yang et al., 2012). Furthermore, Metzler-Zebeli et al. (2013) found that the expression of monocarboxylate transporter 1 (***MCT1***) and Na^+/K^+ ATPase pump subunit α1 (*Na^+/K^+ ATPase*) in the rumen epithelium of goats were strongly tied to ruminal pH values by using regression analyses. Despite the underlying mechanisms not being completely clear, these experiments, together with previous studies in vitro (Fejes-Tóth et al., 1998; Cuff et al., 2002), showed that SCFA and pH could affect the mRNA expression of SCFA transporters. However, the majority of previous studies were conducted using other animals or tissues, such as rabbit cortical collecting duct and human colonic epithelium; therefore, more

Received December 5, 2013.
Accepted May 7, 2014.
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investigation is necessary in the rumen epithelium of goats.

We hypothesized that feeding more concentrate would upregulate the expression of genes involved in the SCFA absorption and that dietary effects on the expression of genes were associated with ruminal SCFA and pH. The current experiments therefore focused on in vivo effects of diet [low concentrate (**LC**; 10% concentrate) vs. medium concentrate (**MC**; 35% concentrate)] and in vitro effects of SCFA concentration and pH value (acidified by HCl) on mRNA expression of genes related to SCFA absorption 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.

Experimental Design and Goat Management

Twelve goats (Boer × Yangtze River Delta White), aged 4 mo at the commencement of the experiment, were used for the feeding trial. The treatments contained concentrate at 10% (LC) and 35% (MC) of dietary DM. The composition of the MC and LC diets is presented in Table 1. Replacing part (almost 30%) of guinea grass with corn meal increased the dietary NFC concentration from 14.7 to 31.3%, and decreased the dietary NDF concentration by almost 17%. The concentration of CP was similar across treatments, averaging 10.5%. Goats were placed in individual pens (1.2 × 1.0 m) with free access to water. Goats were fed in 2 equal portions at 0800 and 1700 h daily, and the amounts of feed offered and refused by each goat was recorded daily throughout the experiment. The amount of feed supplied was adjusted to ensure approximately 25% orts. Adaptation to the diets was carried out over 10 d, and goats received their respective treatment for at least 21 d before slaughter. All goats were slaughtered 6 h after feeding in the morning at a local slaughterhouse.

Table 1. Composition of diets and DMI

Item	Treatment[1]	
	LC	MC
Ingredient (% of DM)		
Guinea grass	90	65
Corn meal	0	25
Soybean meal	8	8
Mineral and vitamin supplement[2]	1	1
NaCl	0.25	0.25
$CaHPO_4$	0.75	0.75
Chemical composition		
DM (%)	91.4	89.9
CP (% of DM)	10.3	10.7
Crude fat (% of DM)	3.5	3.4
Ash (% of DM)	4.7	3.9
NDF (% of DM)	66.8	50.7
NFC[3] (% of DM)	14.7	31.3
ME (MJ/kg of DM)	8.9	10.1
Intake		
Total DMI (g/d)	431.2 ± 9.8	487.4 ± 6.5*

[1]LC = low concentrate; MC = medium concentrate.

[2]Contained 16% calcium carbonate, 102 g of Zn/kg, 47 g of Mn/kg, 26 g of Cu/kg, 1,140 mg of I/kg, 500 mg of Se/kg, 340 mg of Co/kg, 17,167,380 IU of vitamin A/kg, 858,370 IU of vitamin D/kg, and 23,605 IU of vitamin E/kg.

[3]NFC = 100 − (NDF + CP + crude fat + ash).

*Different from LC ($P < 0.05$).

Feed was sampled at d 7 and 21 for chemical analysis. The samples of the feed refusals were collected for DM determination on the same day. Dry matter, ash, CP, and crude fat contents were determined as in our previous work (Malhi et al., 2013). Concentration of NDF was determined using amylase and sodium sulfite (Van Soest et al., 1991).

Sample Collection

Immediately after slaughter, subsamples of ruminal fluid were collected in equal portions from the atrium ruminis, ventral rumen, caudal dorsal, and caudal ventral regions separately and then combined to form 1 sample. The ruminal fluid (30 mL) was strained through 3 layers of cheesecloth and immediately subjected to pH measurement. Thereafter, 5% $HgCl_2$ solution (1.5 mL) was added, and the sample was stored at −20°C. Rumen tissue from the ventral blind sac 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. Epithelium for the cell culture was transported in ice-cold PBS to the laboratory. Epithelium for extracting RNA was separated from the muscle layers and transferred into liquid N within 5 min and stored at −80°C.

Cell Culture

Rumen epithelial cells were isolated from the rumen epithelium of 8 healthy goats (Boer × Yangtze River Delta White, aged 6–12 mo; both sexes) with 0.25% trypsin as described previously (Gálfi et al., 1981). Viability of the cells was confirmed by staining with trypan blue, and the cell density was adjusted to × 1,000,000 cells/mL in high-glucose (4,500 mg/L) Dulbecco's modified Eagle's medium (DMEM; Life Technologies Inc., Gibco/Brl Division, Grand Island,

NY) with 10% fetal bovine serum (Zhejiang Tianhang Biological Technology Co. Ltd., Hangzhou, China), 2 m*M* L-glutamine (Sunshine Chemical Co. Ltd., Nanjing, China), 100 U/mL of penicillin, and 100 mg/mL of streptomycin. The cells were seeded in 25-cm^2 plastic tissue culture flasks (Corning Inc., Corning, NY) and incubated at 37°C in an incubator (5% CO_2) for 24 h. Thereafter, the cells were allocated across treatments in the following manner: pH 7.4 (control; n = 8; 720 kPa) or 6.8 (n = 8; 720 kPa) for 24 h, or 20 m*M* SCFA at pH 7.4 (n = 8; 820 kPa) or 6.8 (n = 8; 820 kPa) for 24 h. After that, the cells were scraped off the flasks and transferred to −80°C until analyzed for mRNA expression. The mixture of SCFA contained 12 m*M* sodium acetate, 5 m*M* sodium propionate, and 3 m*M* sodium butyrate (Merck KGaA, Darmstadt, Germany). In the pH 6.8 group, the medium was acidified with 1 *N* HCl.

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 gas chromatograph HP6890N (Agilent Technologies Inc., New Castle, DE) as described before (Yang et al., 2012).

RNA Extraction and Real-Time Quantitative PCR

Total RNA extraction was carried out as described before (Yang et al., 2012). Complementary DNA was synthesized by using a random hexamer primer (Invitrogen, Shanghai, China) and M-MLV-reverse transcriptase according to the manufacturer's instructions (MBI Fermentas Inc., Burlington, ON, Canada). The primers for *NHE1*, *NHE2*, *NHE3*, vacuolar H^+ ATPase subunit B (***vH$^+$ ATPase***), and *MCT1* were used as described in the published literature (Etschmann et al., 2006; Graham et al., 2007; Albrecht et al., 2008). The primers for the others were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). The PCR products of the newly designed primers were validated by DNA sequencing. The target genes of interest and their respective sources and primer sequences are listed in Table 2. The primers were all synthesized by Shanghai Invitrogen Biological Co. (Shanghai, China).

Determination of relative mRNA expression was performed with real-time quantitative PCR by using the MyiQ2 2-color real-time quantitative PCR detection system (Bio-Rad Laboratories Inc., Hercules, CA). Real-time PCR was carried out in a total volume of 20 μL containing 1× iQ SYBR Green Supermix (Bio-Rad

Table 2. Primers for real-time PCR

Gene[1]	Primer sequence, 5′ to 3′ [2]	Source[3]	Size (bp)
GAPDH	TTGTCTCCTGCGACTTCA CCACCACCCTGTTACTGTT	HM043737.1	135
DRA	TTTAAAGTCCTAGAGTCCGTA CGCTGATTTATTTCTTTAACCAC	BC134586.1	113
PAT1	CCTTGAGGCACGGCTAC GCACCAGACTCCGAGACATA	BC123616.1	123
AE2	AGCAGCAACAACCTGGAGT GGTGAAACGGGAGACGAA	NM_001205664.1	123
MCT1	ACCAGTTTTAGGTCGTCTCA GGCTTCTCAGCAACATCTACA	Somers et al. (2006)	207
MCT4	GTTTGGGATAGGCTACAGTGACACA GCAGCCAAAGCGATTCACA	NM_001109980.1	106
NHE1	CCTCTACAGCTACATGGCCTAC GGGAGATGTTGGCTTCCA	Etschmann et al. (2006)	113
NHE2	TTGGAGAGTCCCTGCTGAAC GGCCGTGATGTAGGACAAAT	Graham et al. (2007)	257
NHE3	AGCTACGTGGCCGAGGG AGACAGAGGCCTCCACGGT	Etschmann et al. (2006)	121
vH$^+$ ATPase	TTTTATTGAACAAGAAGCCAATGA GATTCATCAAATTGGACATCTGAA	Albrecht et al. (2008)	182
Na$^+$/K$^+$ ATPase	TGAGCATCCCAGTGTTGT CCTTGTCCAGATACTTCCT	NM_001076798.1	122

[1] *GAPDH* = glyceraldehyde 3 phosphate dehydrogenase; *DRA* = downregulated in adenoma; *PAT1* = putative anion transporter 1; *AE2* = anion exchanger 2; *MCT1* = monocarboxylic acid transporter 1; *MCT4* = monocarboxylic acid transporter 4; *NHE1* = Na^+/H^+ exchanger 1; *NHE2* = Na^+/H^+ exchanger 2; *NHE3* = Na^+/H^+ exchanger 3; *vH$^+$ ATPase* = vacuolar H^+ ATPase subunit B; *Na$^+$/K$^+$ ATPase* = Na^+/K^+ ATPase pump subunit α1.

[2] The first primer listed for each gene is the forward primer and the second primer is the reverse primer.

[3] The reference sequence number is given for primers whose source is the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

Laboratories Inc.), a mixture of forward and reverse primers (500 n*M* each), cDNA template (1 ng), and sterile water for volume adjustment. An initial cycle for 30 s at 95°C was used to denature the cDNA. This was followed with 45 PCR cycles consisting of denaturation at 95°C for 5 s and primer annealing and extension at 60°C for 30 s. After all PCR analyses, a melting curve analysis was carried out. All investigated PCR products showed only single melting peaks. Before the performance of PCR for experimental samples, the amplification efficiencies of all primers were calculated by using a standard dilution series. The efficiencies of all the primers used were between 97 and 101%. All samples were analyzed in triplicate. These experiments were repeated twice with similar results. Gene expression was normalized to *GAPDH* ($\Delta Ct = Ct_{Target} - Ct_{GAPDH}$, where Ct = cycle threshold). The average ΔCT value of the control group (LC group in vivo experiment and pH-7.4 group in vitro experiment) was then used as a reference value to calculate ΔΔCt. The relative expression values were calculated by using the following formula: relative expression $= 2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001).

Statistical Analyses

All data were expressed as means ± standard error. Differences were considered significant when $P < 0.05$, as tested by the independent sample *t*-test (in vivo data). The general linear model 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

Ruminal Fermentation

The goats in the MC group had lower ruminal pH than goats in the LC group (6.63 vs.7.03; $P = 0.027$; Table 3). In agreement with ruminal pH results, total ruminal SCFA increased in the MC group compared with the LC group. Of all 6 SCFA analyzed, feeding MC increased ($P < 0.05$) the concentration of propionate by 49%, butyrate by 152%, and isobutyrate 45%, respectively.

Gene Expression In Vivo

All of the genes evaluated in ruminal tissue were affected by the dietary treatment ($P < 0.05$; Table 4). Gene expression of 3 candidates for $SCFA^-/HCO_3^-$ exchangers, namely downregulated in adenoma (***DRA***), putative anion transporter 1 (***PAT1***), and anion exchanger 2 (***AE2***) were upregulated to a greater extent in the MC group compared with the LC group. Further, the mRNA abundances of *MCT1* and *MCT4*, which were cotransporters of monocarboxylate anions and protons, were higher in the MC group than in the LC group.

The expression of genes by which the rumen epithelial cells maintain intracellular pH, including *NHE1*, *NHE2*, and *NHE3* were also evaluated by quantitative PCR. Their expressions in the MC group were enhanced relative to the LC group.

In addition, we examined the mRNA amount of 2 ATPase, which are also involved in intracellular pH (**pHi**) regulation, including *vH^+ ATPase* and *Na^+/K^+ ATPase*. The expressions of *vH^+ ATPase* and *Na^+/K^+*

Table 3. Effects of dietary concentrate level on ruminal short-chain FA (SCFA) and pH in goats 6 h after feeding[1]

	Treatment			
Item	LC	MC	SEM	*P*-value
pH	7.03	6.63	0.10	0.027
Acetate (m*M*)	37.3	46.1	3.41	0.23
Propionate (m*M*)	12.6	18.8	1.61	0.030
Butyrate (m*M*)	4.8	12.2	2.25	0.040
Isobutyrate (m*M*)	1.0	1.5	0.11	0.037
Valerate (m*M*)	1.0	1.6	0.15	0.12
Isovalerate (m*M*)	1.2	1.8	0.17	0.078
Total SCFA (m*M*)	58.0	81.9	6.86	0.037

[1]Goats received the low-concentrate (LC) or medium-concentrate (MC) diet for at least 21 d before slaughter.

Table 4. Effect of dietary concentrate level on the relative expression of genes related to short-chain FA absorption and intracellular pH regulation in ruminal epithelium of goats[1]

	Treatment			
Gene[2]	LC	MC	SEM	*P*-value
DRA	1.14	3.24	0.44	0.009
PAT1	1.15	4.51	0.66	0.010
AE2	1.21	3.01	0.35	0.003
MCT1	0.85	2.65	0.32	0.001
MCT4	1.25	5.49	0.88	0.019
NHE1	1.06	2.47	0.30	0.016
NHE2	1.07	3.30	0.40	0.001
NHE3	1.16	3.50	0.50	0.017
vH^+ ATPase	1.20	6.86	1.06	0.006
Na^+/K^+ ATPase	1.24	5.86	0.87	0.006

[1]Goats received the low-concentrate (LC) or medium-concentrate (MC) diet for at least 21 d before slaughter.

[2]*DRA* = downregulated in adenoma; *PAT1* = putative anion transporter 1; *AE2* = anion exchanger 2; *MCT1* = monocarboxylic acid transporter 1; *MCT4* = monocarboxylic acid transporter 4; *NHE1* = Na^+/H^+ exchanger 1; *NHE2* = Na^+/H^+ exchanger 2; *NHE3* = Na^+/H^+ exchanger 3; *vH^+ ATPase* = vacuolar H^+ ATPase subunit B; *Na^+/K^+ ATPase* = Na^+/K^+ ATPase pump subunit α1.

ATPase mRNA in the MC group were increased compared with those in the LC group.

Gene Expression In Vitro

A decrease in pH from 7.4 to 6.8 increased ($P < 0.001$) *MCT1* mRNA expression after 24 h of culture (Figure 1). Conversely, the mRNA expression of *AE2* was downregulated ($P < 0.001$) by the addition of acid. The presence of SCFA (20 m*M*) in the medium increased *DRA*, *PAT1*, *AE2*, *MCT1*, and Na^+/K^+ *ATPase* mRNA expression ($P < 0.001$). When comparing this increase at pH 7.4 with and without SCFA, it was by 117, 993, 199, 54, and 67%, respectively. In contrast, the expression of vH^+ *ATPase B subunit* was decreased ($P = 0.002$) by SCFA. The expression of *DRA* was elevated by synergistic effects of increased SCFA concentration and decreased pH, similar to that of *MCT1* (interaction: $P < 0.01$; Figure 1). No interaction was observed between adding SCFA and low pH for mRNA expression of *MCT4*, *PAT1*, *AE2*, vH^+ *ATPase B subunit*, and Na^+/K^+ *ATPase* (interaction: $P > 0.05$). Messenger RNA expression of *MCT4* was not changed by treatment.

DISCUSSION

Ruminal Fermentation

In the present study, different diets induced differences in ruminal fermentation. Ruminal total SCFA concentration was 40% greater and pH was 0.4 U less in goats in the MC compared with the LC group when measured 6 h after the last feeding. This result is in agreement with previous reports that suggested that ruminal pH decreased and total SCFA concentrations increased as dietary concentrate-to-forage ratio (Penner et al., 2009) or NFC level (Valadares et al., 1999) increased.

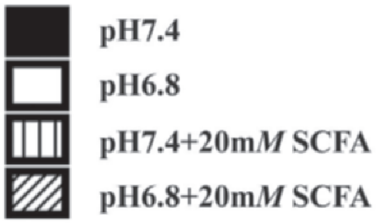
Effects of Diet on the Expression of Genes Related to SCFA Absorption

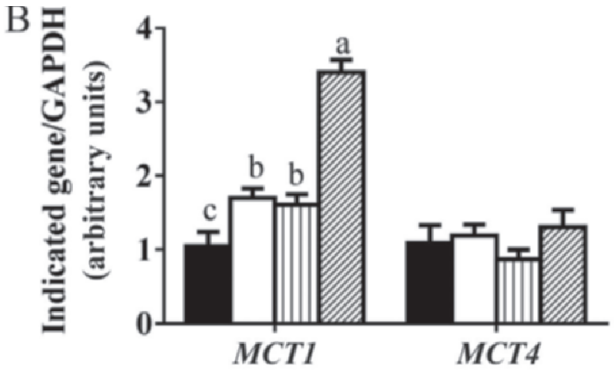
Previous studies in sheep (Doreau et al., 1997) and cows (Sehested et al., 2000) revealed that feeding highly fermentable diets not only caused enhanced ruminal fermentation, but also increased SCFA absorption. Sehested et al. (2000) reported that the rumen epithelium of cows fed additional concentrate had an increased rate of butyrate transport, measured in Ussing chambers, despite unchanged absorptive surface area of the rumen papillae. One possible explanation for the observed change is that the SCFA transport system of epithelial cells was enhanced by feeding additional concentrate. Kuzinski and Röntgen (2011) reported that the rumen epithelium of sheep fed additional concentrate had increased *MCT1* expression. Similarly, a report in calves showed that the gene expression of *DRA* was upregulated by concentrate feeding (Connor et al., 2010). These reports suggested that diets could influence the SCFA absorption capacity of rumen by regulating the transcription of transporters. In the present experiment, mRNA expression of the SCFA transporters (*MCT1*, *MCT4*, *DRA*, *PAT1*, and *AE2*) were all enhanced in goats in the MC compared with the LC group. These data lead to the speculation that the SCFA absorption capacity of the rumen increased when goats were fed the MC diet. Besides, the expression of genes (*NHE1*, *NHE2*, *NHE3*, vH^+ *ATPase*, and Na^+/K^+ *ATPase*) that are responsible for maintaining pHi were also upregulated to a greater extent in the MC group compared with the LC group. In agreement with our findings, Yang et al. (2012) demonstrated the mRNA expression of *NHE1* and *NHE3* in the rumen epithelium was greater for goats receiving additional concentrate. The results that expression of genes for SCFA transporters and intracellular pH regulators were both increased is of significant physiological importance, because enhanced SCFA uptake increases the intracellular proton load (Aschenbach et al., 2011), which must be excreted from the epithelial cells to maintain a constant pHi.

Effects of SCFA and pH on the Expression of Genes Related to SCFA Absorption

During the last decade, a surge of studies were done to determine dietary effects on the transcription changes in proteins involved in SCFA absorption (Penner et al., 2009; Kuzinski and Röntgen, 2011; Steele et al., 2011); however, most of those studies looked for individual SCFA transporter genes without further investigation into the influencing factors. The results of previous studies showed that luminal factors, especially SCFA and pH, can work as the modulators of gene transcription (DeCastro et al., 2005; Li and Li, 2006; Yang et al., 2012). Furthermore, we observed marked differences in the ruminal fermentation measurements, including SCFA concentration and ruminal pH in the vivo experiment. Based on the above information, we assumed that SCFA and pH might affect the transcription of genes related to SCFA absorption. To test the assumption, we conducted a trial in vitro.

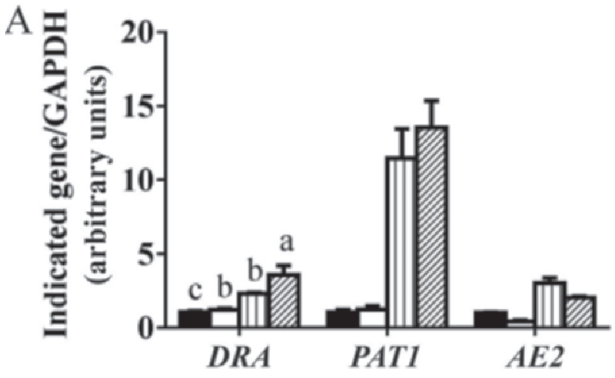
In the present experiments in vitro, mRNA expression of *DRA*, *PAT1*, *AE2*, *MCT1*, and Na^+/K^+ *ATPase* was stimulated by either reduced pH or increased SCFA concentration after 24 h of treatment. In accordance with our results, previous studies had shown that SCFA, especially butyrate, stimulated *DRA* (Alrefai et





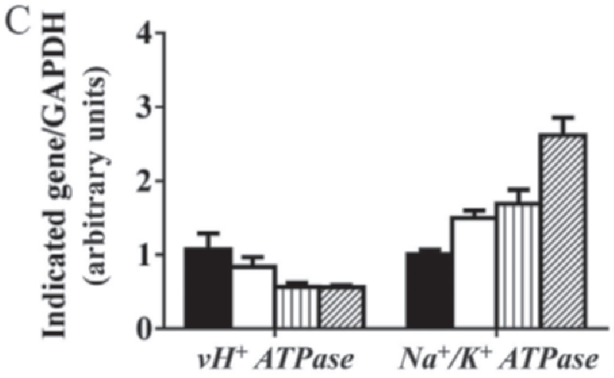
Effects on *MCT1* and *MCT4* expression:

	MCT1	*MCT4*
pH	$P < 0.001$	$P = 0.18$
SCFA	$P < 0.001$	$P = 0.78$
pH*SCFA	$P = 0.003$	$P = 0.40$



Effects on *DRA*, *PAT1*, and *AE2* expression:

	DRA	*PAT1*	*AE2*
pH	$P = 0.048$	$P = 0.39$	$P = 0.001$
SCFA	$P < 0.001$	$P < 0.001$	$P < 0.001$
pH*SCFA	$P = 0.003$	$P = 0.47$	$P = 0.27$



Effects on *vH⁺ ATPase* and *Na⁺/K⁺ ATPase* expression:

	vH⁺ ATPase	*Na⁺/K⁺ ATPase*
pH	$P = 0.22$	$P = 0.001$
SCFA	$P = 0.002$	$P < 0.001$
pH*SCFA	$P = 0.46$	$P = 0.22$

Figure 1. In vitro effects of acid (pH 6.8), short-chain FA (SCFA; 20 m*M*), and acid (pH 6.8) + SCFA (20 m*M*) on gene expression in rumen epithelial cells of goats after cells were incubated for 24 h. In groups with pH 6.8 treatment, the medium was acidified with 1 *N* HCl. The 20 m*M* SCFA contained 12 m*M* sodium acetate, 5 m*M* sodium propionate, and 3 m*M* sodium butyrate. The levels of gene expression were quantified with real-time PCR in comparison with GAPDH. Values are mean ± SEM (n = 8). Values within a group without a common letter (a–c) are significantly different ($P < 0.05$). *DRA* = downregulated in adenoma; *PAT1* = putative anion transporter 1; *AE2* = anion exchanger 2; *MCT1* and *MCT4* = monocarboxylate transporters 1 and 4, respectively; *vH⁺ ATPase* = vacuolar H+ ATPase subunit B; *Na⁺/K⁺ ATPase* = Na^+/K^+ ATPase pump subunit α1.

al., 2007) and *MCT1* (Cuff et al., 2002; Borthakur et al., 2008) expression in certain types of cells (LS174T cells, AA/C1 cells, and Caco-2 cells). Our results are also in agreement with the previous observation in vivo that showed negative relationships of *MCT1* and *Na⁺/K⁺ ATPase* expression in the ruminal epithelium of

goats with decreasing luminal pH values, suggesting greater mRNA expression at lower pH (Metzler-Zebeli et al., 2013). In addition, our previous work demonstrated that low pH and SCFA could enhance mRNA expression of *NHE1* and *NHE3* after 24 h of incubation (Yang et al., 2012). These previous data together with the present results from the in vitro study suggest that the dietary effects on the rumen epithelial mRNA expression of those genes (*DRA*, *PAT1*, *AE2*, *MCT1*, *Na^+/K^+ ATPase*, *NHE1*, and *NHE3*) were associated with ruminal SCFA and ruminal pH. The effects of SCFA and pH on transporter expression is of significant physiological importance. Low pH in combination with greater SCFA concentrations imposed challenges on the SCFA absorption and pHi regulation in epithelial cells, which could be alleviated by the enhanced expression of these proteins. Our experiment also indicated a synergistic effect of low pH and adding SCFA on the expression of *DRA* and *MCT1*. These results further showed that reduced pH or increased SCFA concentration could not only act as independent factors, but when combined, they could also synergistically induce mRNA expression.

In this study, we found that the mRNA expression of *AE2* was downregulated by the addition of acid. This result is in agreement with a study showing that high extracellular H^+ concentration led to a decrease in *AE2* mRNA expression in isolated rabbit collecting duct cells (Fejes-Tóth et al., 1998). Meanwhile, we also found that both reduced pH and SCFA could not promote the expression of *vH^+ ATPase* and *MCT4* in vitro. In addition, our previous experiments showed that neither SCFA nor pH affected the expression of *NHE2* mRNA (Yang et al., 2012). In the present experiments, the transcription changes of these genes (*MCT4*, *vH^+ ATPase*, and *NHE2*) in vivo were not aligned with the changes in vitro. One may, thus, speculate that other factors, such as hypoxia (Ullah et al., 2006) and epidermal growth factor (Xu et al., 2001), could have influenced the transcription of the latter genes. Furthermore, the effects of those factors cannot be excluded in the current vivo experiment. It is possible that the dietary treatment imposed in the current study caused different physiological and pathological changes, apart from ruminal SCFA and pH, which affected the gene expression of *MCT4*, *vH^+ ATPase*, and *NHE2*.

CONCLUSIONS

Increased dietary concentrate led to enhanced SCFA production and reduced ruminal pH. In parallel, the expression of genes related to SCFA absorption (*DRA*, *PAT1*, *AE2*, *MCT1*, *MCT4*, *NHE1*, *NHE2*, *NHE3*, *Na^+/K^+ ATPase*, and *vH^+ ATPase*) was enhanced according to the dietary concentrate. Furthermore, the results of the in vitro experiment indicated that dietary modulation of the expression of genes involved in SCFA absorption in the rumen epithelium are associated with the ruminal SCFA and ruminal pH. The effects of SCFA and pH on gene transcription offer new insights into the molecular adaptation of the rumen epithelium to increased dietary concentrate.

ACKNOWLEDGMENTS

This work was supported by the Key Basic Research Program (973 Program) in China (No. 2011CB100801), the National Natural Science Foundation of China (Beijing, China; No. 30771568), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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