Rumen epithelial adaptation to ruminal acidosis in lactating cattle involves the coordinated expression of insulin-like growth factor-binding proteins and a cholesterolgenic enzyme

M. A. Steele, L. Dionissopoulos, O. AlZahal, J. Doelman, and B. W. McBride¹

Department of Animal and Poultry Science, University of Guelph, Guelph, N1G 2W1, Canada

ABSTRACT

The objective of this study was to characterize the mRNA expression of metabolic and proliferative genes in the rumen epithelium during ruminal acidosis. To meet our objectives, 16 rumen-fistulated, lactating Holstein dairy cattle (618 ± 35 kg of body weight, 221 ± 32 d in milk) were used in a randomized complete block design. All cattle were fed a high-forage diet (HF; 88.9% of dry matter) for 5 wk before the experiment. After the baseline week (wk 0), half of the cattle were randomly assigned and transitioned to a high-concentrate diet (HC; 62.2% of dry matter) which was fed for 3 wk (wk 1, 2, and 3). For the last 48 h of each week, continuous ruminal pH, short-chain fatty acids, and plasma β -hydroxybutyrate were assessed, followed by a rumen papillae biopsy. Milk production was higher in HC cattle compared with HF during wk 1, 2, and 3 (17.4 ± 0.5 vs. 23.4 ± 0.9 kg/d, respectively); however, the mean ruminal pH was decreased (5.75 ± 0.03 vs. 6.30 ± 0.02). The HC cattle spent more time below pH 5.6 (594 ± 54 vs. 3 ± 3 min/d) and displayed greater concentrations of ruminal butyrate (15.8 ± 0.9 vs. 10.2 ± 0.4 mmol) and plasma β -hydroxybutyrate ($1,036 \pm 63$ vs. 778 ± 20 μ M) compared with the HF cattle. The mRNA expression of genes involved in ketogenesis (*HMGCS2* and *PPARA*) and short-chain fatty acid transport (*MCT1*) was unchanged by treatment. However, a downregulation in *HMGCS1* (0.72 ± 0.09), one of the cholesterol biosynthesis genes, was observed in HC cattle during wk 1 of the grain challenge. In addition, the relative mRNA expression value of insulin-like growth factor-binding protein 3 was lower (0.78 ± 0.06), whereas insulin-like growth factor-binding protein 5 was higher (1.79 ± 0.15) in HC compared with HF cattle. These results suggest that grain-induced ruminal acidosis alters the mRNA expression of IGF-binding proteins and a cholesterolgenic enzyme in the rumen epithelium of lactating dairy cattle.

Key words: rumen epithelium, ruminal acidosis, gene expression

INTRODUCTION

Lactating dairy cattle rely on diets rich in rapidly fermentable carbohydrates (high-grain) to increase dietary energy and, thus, production efficiency. When cattle are transitioned abruptly to a high-grain diet, such as during the periparturient period, the rate of short-chain fatty acid (SCFA) production often exceeds the rate of buffering and absorption through the rumen epithelium (RE), making the cow more susceptible to developing the digestive disorder termed ruminal acidosis (Owens et al., 1998). Subacute ruminal acidosis, a common occurrence among commercial dairy operations (Oetzel et al., 1999), is associated with depressed feed intake and production, rumenitis, liver abscesses, and whole-animal inflammatory response, thereby causing significant economic (Stone, 1999) and animal welfare issues in ruminant production systems (Plaizier et al., 2008).

The rumen adapts to highly fermentable diets by increasing the size of rumen papillae and, thus, the surface area for absorption (Goodlad, 1981; Odongo et al., 2006), yet the molecular signaling mechanisms orchestrating the proliferative changes are still unknown. Among candidate mechanisms, IGF-1 is thought to be involved in RE growth, as it induces RE cell proliferation in vitro (Baldwin, 1999). Cellular events of IGF-1 are modulated by a family of IGF-binding proteins (IGFBP; Firth and Baxter, 2002). In a recent study, the mRNA expression of IGFBP3 and IGFBP6 was downregulated and IGFBP5 was upregulated in the RE of nonlactating cattle during the transition to a high-grain diet (Steele et al., 2011a). It is unknown if cattle respond in a similar fashion during lactation that is accompanied by increased feed intake (Penner et al., 2011) and altered gastrointestinal function (Cripps and Williams, 1975); therefore, more investigation is necessary.

Although the RE is essential for the absorption of fermentation end products, it also plays an important role in the metabolism of SCFA (Gäbel et al., 2002). It

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¹Corresponding author: bmcbride@uoguelph.ca

has been suggested that ruminal SCFA can be absorbed through passive diffusion and facilitated diffusion via H^+ -monocarboxylate transporters (Kirat et al., 2006; Graham et al., 2007). Ketogenesis is the main pathway in the RE, as more ketone bodies are synthesized in the RE compared with the liver in the fed state (Leighton et al., 1983). Short-chain fatty acids can be converted to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) by HMG-CoA synthase (HMGCS2) in the mitochondria, which proceeds to ketogenesis (Meertens et al., 1998). In the cytoplasm, SCFA can be converted to HMG-CoA by HMGCS1, which proceeds to cholesterol biosynthesis through a series of cytosolic enzymes. To our knowledge, no differences in RE ketogenic gene expression have been detected; however, we recently found that cholesterolgenic genes were down-regulated in the RE of nonlactating cows exposed to a grain challenge (Steele et al., 2011b). It is unknown if the expression signature of cholesterolgenic genes is similar in lactating cattle with higher feed intake, and therefore warrants further exploration.

Despite recent progress in our understanding of how the RE adapts to high-grain diets, the molecular mechanisms remain largely uncharacterized and elusive, especially in the lactating dairy cow. We hypothesized that feeding a high-grain diet to induce ruminal acidosis will alter the expression of genes associated with the IGF axis and cholesterol biosynthesis in the RE of lactating dairy cattle. Therefore, the aims of the current study were to induce a prolonged state of ruminal acidosis in lactating dairy cattle and characterize the mRNA expression of candidate genes involved in RE metabolism and proliferation.

MATERIALS AND METHODS

Animals, Experimental Treatments, and Feeding

Sixteen lactating Holstein dairy cattle (618 ± 35 kg of BW, mean $\pm$ SD) in their second lactation (221 ± 32 DIM, mean $\pm$ SD) were used in a randomized complete block design study. Prior to the start of the experiment, cows were grouped according to stage of lactation into 2 blocks ($n = 8$ each) and cows within each block were randomly assigned into 1 of 2 dietary treatments. To minimize the effect of stage of lactation on results, each block was enrolled in the experiment when each reached, on average, the above-specified DIM. All experimental procedures were approved by the University of Guelph Animal Care Committee in accordance with the Canadian Council on Animal Care (1993). Cattle were fitted with a rumen fistula during the dry period before their second lactation as described by Duffield (1999), and housed in a tie-stall facility at the Pon-

sonby Dairy Research Station (University of Guelph, Guelph, Ontario, Canada). Throughout their lactation, the cattle had unlimited access to fresh water and were milked twice daily in their stalls at 0600 and 1600 h.

Five weeks preceding the experiment, all cattle were gradually transitioned to a high-forage diet (HF; 88.9% hay, 6.7% grain pellet, and 4.4% supplement, % of DM; Table 1) over 1 wk. The grain mix comprising 40% wheat, 40% barley, and 20% corn (% of DM), was fed in equal allotments at 0800, 1200, and 1600 h. The protein supplement and hay were fed at 0800 and 1600 h, also in equal allotments. Throughout the experiment, the concentrate portion was fed in plastic trays and if animals did not consume it within 60 min post-feeding, the remainder was introduced into the rumen through the fistula. Dry matter intake was calculated on a daily basis for each cow throughout the experiment using previously described methodology (Keunen et al., 2002). The average intake for each cow during the baseline week (wk 0) was fed for the remaining 3 wk (wk 1, 2, and 3). At the end of wk 0, the cattle were randomly assigned to the control group (HF diet) or transitioned to a high-concentrate diet (HC; Table 1). Treatment cattle were transitioned to the HC diet in equal increments over 5 d, which was fed until the end of experimental wk 3.

DMI, Milk Production, and Milk Components

All physiological measurements were collected during the final 2 d of each week (wk 0, 1, 2, and 3). Daily DMI measurements during the final 2 d of each week were used in the analysis. Milk production was also recorded on a daily basis and milk samples were collected from each milking (0600 and 1600 h) for the last 2 d of each experiment week and analyzed for milk composition at the CanWest DHI Milk Laboratory (Guelph, Ontario, Canada) using infrared spectroscopy (Milkoscan 4000; Foss Electric, Hillerød, Denmark). The yield of milk protein and milk fat produced during the last 2 d was calculated for each week.

Ruminal pH, Ruminal SCFA, and Plasma BHBA

Ruminal pH probes were assembled as described by AlZahal et al. (2007a) and calibrated before weekly recordings. Ruminal pH was measured every min of the last 48 h of each experimental week (wk 0, 1, 2, and 3) using a pH recording system and methodology described previously by our research group (AlZahal et al., 2007b). To characterize how daily ruminal SCFA were altered by treatment, rumen fluid samples were collected at 1600 h for the last 2 d of each experimental week. Rumen contents were collected from the ventral

Table 1. Ingredient composition and chemical analyses of high-forage (HF) and high-concentrate (HC) diets

Composition	HF	HC
Ingredient, % of DM		
Supplement ¹	4.4	4.4
Grain mix	6.7	57.8
Grass hay	88.9	37.8
Chemical composition, % of DM		
DM, %	88.2	88.2
CP (N $\times$ 6.25)	16.2	14.5
ADF	31.8	17.6
NDF	46.9	32.3
Lignin	4.0	3.0
Ether extract	2.2	2.5
Ash	10.6	7.1
NFC ²	27.9	46.0
Starch ³	7.4	32.1
NE _L , ⁴ Mcal/kg	1.4	1.7

¹Contained (% as-fed basis): dried distillers grains with solubles, 34.6; 48% soybean meal, 33.4; salt, 9.2; monocalcium phosphate, 8.2; calcium carbonate (limestone), 5.1; molasses, 4; tallow, Organic Ruminant Micro Premix (Floradale Feed Mill Ltd., Floradale, ON, Canada), 2.8; magnesium oxide, 2.0; sulfur flour (99.5%), 0.7; and Sel-Plex 2000 (Alltech Inc., Nicholasville, KY), 0.03.

²NFC = 100 – (NDF + CP + ether extract + ash).

³Analyzed according to Hall (2000).

⁴Estimated using CPM-Dairy version 3.0.8 (Miner Institute, Chazy, NY) using the chemical analysis of feed ingredients.

sac and squeezed through 3 layers of cheesecloth to recover rumen fluid samples, which were immediately frozen at -20°C until processing. The concentration of SCFA in rumen fluid samples was quantified in duplicate by gas chromatography (Steele et al., 2011a).

To monitor blood BHBA concentration, a 14-gauge catheter (Becton Dickinson Angiocath; Becton, Dickinson and Co., Franklin Lakes, NJ) was inserted into 1 jugular vein of each cow under local anesthesia in the morning before blood collection. Blood samples were collected at 1600 h during the final 2 d of each experiment week. Immediately after collection, blood samples were put on ice, centrifuged at $3000 \times g$ for 15 min and frozen at -20°C until analysis. The concentration of BHBA in plasma was determined in duplicate using an enzymatic kit (kit 310-A; Sigma, Oakville, ON, Canada) following previously described methods (Williamson et al., 1962).

Rumen Papillae Biopsies, RNA Collection, and Quality Assessment

The ventral sac of the rumen was chosen as a biopsy site because it has the highest capillary blood flow per unit weight mucosa of any location within the rumen (Von Engelhardt and Hales, 1977). Rumen papillae were biopsied from the ruminal ventral sac at the end of wk 0, 1, 2, and 3 (0700 h) based upon previously developed methodology (Steele et al., 2011b). In brief,

the reticulorumen contents were partially evacuated to facilitate the retraction of the ventral sac. Rumen papillae were excised (150 mg) using surgical scissors each week from previously unbiopsied sites, washed 20 times in ice-cold PBS (pH 7.4), snap-frozen in liquid nitrogen, and then stored at -80°C until RNA isolation.

Total RNA was isolated from approximately 100 mg of tissue using an RNeasy midi kit (Qiagen Inc., Mississauga, ON, Canada). The concentration was determined using a NanoDrop spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE) and the 260:280 ratio was determined for all samples to be between 1.9 and 2.1. The RNA was then treated with DNase (Invitrogen Canada Inc., Burlington, ON, Canada) before assessing the quality using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA) and the RNA 6000 Nano kit (Caliper Life Sciences Inc., Mountain View, CA).

Real-Time Reverse-Transcription Quantitative PCR

To assess mRNA expression of target proliferative and metabolic genes identified from a previous microarray experiment [IGF-binding protein 3 (*IGFBP3*), IGF-binding protein 5 (*IGFBP5*), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (*HMGCS1*), and farnesyl diphosphate synthase (*FDPS*); Steele et al., 2011a,b] and the literature [monocarboxylate transporter 1 (*MCT1*), peroxisome proliferator-activated receptor alpha (*PPARA*), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (*HMGCS2*), and sterol regulatory element-binding protein 1 and 2 (*SREBP1*, *SREBP2*)], quantitative PCR (qPCR) was performed. After RNA isolation and quality assessment, samples (500 ng each) were reverse-transcribed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). The Power SYBR Green PCR master mix (Applied Biosystems) was used to perform qPCR in duplicate using an ABI Prism 7000 (Applied Biosystems). The sequences, amplicon size, and primer efficiencies of primers are presented in Table 2. Exon-spanning primers for target genes were designed using Primer Express 3.0 (Applied Biosystems) from bovine sequences listed in GenBank (National Center for Biotechnology Information, Bethesda, MD). The amplicon of all primers designed for this study were sequenced and verified using BLASTN in NCBI (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Nucleotides>) and dissociation curves were generated at the end of amplification to verify the presence of a single product. The standard curve method with 5 dilutions ranging from 1:5 to 1:3125 was used to calculate the efficiency of qPCR amplification. Based upon previous experimental protocols (Steele et al., 2011b), glyceraldehyde-3-phosphate dehydrogenase

(*GAPDH*) was determined to be the most stable house-keeping gene in the rumen papillae and was, therefore, used in this study. For each week, the relative mRNA expression of genes was calculated using the inverse of qPCR efficiency raised to delta cycle threshold (Ct) (Pfaffl, 2001). The HF cattle were pooled for each week to create a control value to which each HC sample was normalized for determination of relative mRNA expression as described previously (Xue et al., 2010).

Statistical Analysis of Physiological and qPCR Measurements

Average weekly milk production, ruminal pH characteristics, ruminal SCFA, and blood BHBA were analyzed using the PROC MIXED of SAS (SAS Institute, 2004) using the following model:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + B_k + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the overall mean, D_i is the fixed effect of diet ($i = 1, 2$), T_j is the fixed effect of time or week ($j = 1, \dots, 4$), $(D \times T)_{ij}$ is the effect of the interaction of diet by time ($ij = 1, \dots, 8$), B_k is the fixed effect of block or phase ($k = 1, 2$), and ε_{ijk} is the random residual error. Week of experiment was used as a repeated measurement with cow as the subject. For each analyzed variable, cow was subjected to 5 covariance structures: compound symmetry, heterogeneous compound symmetry, autoregressive order 1, heterogeneous autoregressive order 1, and unstructured covariance structure. The covariance structure that gave the smallest Bayesian information criterion was used. Because all cows received the HF diet during wk 0 and received either the HF or the HC diet during wk 1, 2, and 3, the difference between dietary treatments was represented by the interaction between treatment and time. Helmert contrasts compare the mean of each level with the overall mean of the remaining or subsequent levels and were used to evaluate significant changes due to treatment. The first contrast compared wk 0 and average of wk 1, 2, and 3 in the HF group to that in the HC group. The second contrast compared the difference between wk 1 and average of wk 2 and 3 between treatments. The third contrast compared the difference between wk 2 and 3 between treatments. The contrasts were generated using the ESTIMATE statement of SAS. Gene expression values for HC cattle (generated relative to HF cattle) were analyzed using PROC MIXED of SAS with time (week) and block (phase) as fixed effects. Repeated measurement effect was accounted for as discussed previously. T-statistics were conducted to

determine whether HC relative gene expression values were different from 1. The associations between relative gene expression values of HC cattle for specific genes were determined using PROC MIXED of SAS accounting for repeated measurement. An adjusted coefficient of determination was calculated using the GLM of SAS and the model included the adjusted observations as the dependent variables according to St. Pierre (2001).

RESULTS

DMI, Milk Production, Ruminal pH, Ruminal SCFA, and Plasma BHBA

All DMI, milk production, ruminal pH, ruminal SCFA, and plasma BHBA results have been summarized in Table 3. No differences were detected in the Helmert contrast between wk 2 and 3 for all variables and were not reported in Table 3. Dry matter intake was decreased ($P < 0.01$) in HC by 1.7 ± 0.31 kg/d compared with HF cattle. The increased energy density of the diet translated into increased ($P < 0.01$) milk production (17.4 ± 0.5 vs. 23.4 ± 0.9 kg/d) and milk protein yield (559 ± 16 vs. 782 ± 23 g/d) during all 3 wk. The percentage of milk protein was also higher ($P < 0.01$) throughout the experiment in HC cattle compared with HF cattle. Milk fat percentage was decreased in HC cattle ($P < 0.01$), but yield of milk fat was not different between treatments (Table 3).

Dramatic differences in ruminal pH were evident between HC and HF cattle during experimental wk 1, 2, and 3 (Figure 1). Week 1 was marked by the largest change in ruminal pH ($P < 0.01$) as HC cattle displayed 760 ± 71 min/d and $1,139 \pm 57$ min/d below 5.6 and 6.0, respectively. Time below pH thresholds 5.6 and 6.0 decreased from wk 1 to 2 and 3 ($P < 0.01$) in HC cattle, yet was still greater compared with HF cattle ($P < 0.01$). The mean and minimum daily ruminal pH was lower during every week ($P < 0.01$) in the HC cattle compared with the HF cattle. The daily minimal ruminal pH reached levels below pH 5.0 for each experimental week, indicative of a severe state of SARA.

In agreement with ruminal pH results, total SCFA concentrations were greater ($P < 0.01$) in HC compared with HF cattle during wk 1, 2, and 3 (Table 3). The concentrations of all SCFA were greater in HC cattle throughout the experiment, except for acetate and isobutyrate, which remained unchanged. The ratio of propionate to acetate was lower ($P < 0.01$) in HC (1.83) compared with HF (2.96) cattle during wk 1, 2, and 3. Of the dominant SCFA (acetate, propionate, and butyrate), ruminal butyrate displayed the largest proportional increase ($P < 0.01$) due to treatment (6.1

Table 2. Primers for real-time quantitative PCR (qPCR)

Gene	Gene name	Gene ID	Primer ¹	Amplicon size	Eff% ²	Source
<i>HMGCS1</i>	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	XM_002696364.1	F1316-AGAGGATCGGCGTGTTTTCTT	87	93.5	Steele et al. (2011b)
<i>HMGCS2</i>	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2	NM_001045883	R1403-CAGACCCTGGTGTGGCATCT F1179-TCTGGCCCATCACTCTGCC	126	84.0	Steele et al. (2011b)
<i>FDPS</i>	Squalene synthase 1	NM_177497	R1305-AGTGGGGAGCCTGGAGAAGC F362-GGTATCAGAAGCCAGGCATAGG R465-TAGGGCTGCTCCCGACAGTA	103	93.1	Steele et al. (2011b)
<i>MCT1</i>	Monocarboxylic acid transporter 1	NM_001037319.1	F137-CGAGCCGCGTATAACGATACTT R294-GAGAAGCCGATGGAAATGAAAG	157	94.5	
<i>IGFBP3</i>	IGF-binding protein 3	NM_001075549	F855-GCGACAAGAAGGGCTTTTACAA R936-TATCCACACACCAGCAGAAACC	81	85.7	Steele et al. (2011a)
<i>IGFBP5</i>	IGF-binding protein 5	NM_001105327	F701-CTACAAGAGAAAGCAGTGCAAACC R764-TCCACGCACCAGCAGATG	63	94.6	Steele et al. (2011a)
<i>PPARA</i>	Peroxisome proliferator-activated receptor alpha	NM_001034036.1	F342-CGGTGTCCACGCATGTGA R397-TCAGCCGAATCGTTCTCCTAAA	56	91.7	Loor et al. (2005)
<i>SREBP1</i>	Sterol regulatory element-binding protein 1	NM_001113302.1	F143-FCCAGCTGACAGCTCCATTGA R209-TGCGCGCCACAAGGA	67	92.5	Steele et al. (2011b)
<i>SREBP2</i>	Sterol regulatory element-binding protein 2	XM_002687950.1	F147-CTGGCTCCAGGGAGATGAC R266-GCTCTGCAGGTGTGGAAGAC	120	88.0	Steele et al. (2011b)
<i>GAPD</i>	Glyceraldehyde-3-phosphate dehydrogenase	NM_001034034	F276-TGGAAAGGCCATCACCATCT R335-CCCCTTGATGTTGGCAG	60	100.3	Steele et al. (2011b)

¹Exon junctions are underlined.
²The PCR efficiency (Eff%) was calculated as follows: $E = -1 + 10^{(-1/\text{slope})} \times 100$, where E = efficiency; the slope was obtained by the 5 points standard curve with an R² of 0.99.

Figure 1. Mean 24-h rumen pH values for the 2 d of recordings of cattle fed high-concentrate (HC, $n = 8$; black) and high-forage (HF, $n = 8$; gray) diets during experimental wk 0, 1, 2, and 3. The dashed line represents ruminal pH threshold 5.6.

± 1.6 mM). Plasma BHBA was also higher ($P = 0.04$) in HC cattle throughout the experiment compared with HF cattle.

Gene Expression

The relative fold changes between HC and HF are presented in Table 4 with the Helmert contrast between

wk 0 and the average of wk 1, 2, and 3. No differences ($P \geq 0.05$) were detected in the Helmert contrasts between wk 1 and the average of wk 2 and 3 and between wk 2 and 3 for all genes. The mRNA expression was not different between treatments for all genes during the baseline wk 0; however, differential expression was detected in specific genes during wk 1, 2, and 3.

Table 3. The effect of feeding the high-forage (HF) or high-concentrate (HC) diet over time on feed intake, milk production, ruminal pH, ruminal short-chain fatty acids (SCFA), and plasma BHBA¹

Item	HF				HC				SE	<i>P</i> -value ²	
	wk 0	wk 1	wk 2	wk 3	wk 0	wk 1	wk 2	wk 3		0 vs. 123	1 vs. 23
DMI and production											
DMI, kg/d	19.2	19.6	19.8	20.1	19.4	18.1	18.3	18.1	0.7	<0.00	0.42
Fat, %	3.83	4.15	3.93	3.74	3.89	3.56	3.59	3.25	0.20	<0.01	0.40
Fat, kg/d	0.70	0.73	0.68	0.64	0.76	0.81	0.83	0.73	0.05	0.36	0.57
Protein, %	3.07	3.11	3.17	3.22	2.98	3.28	3.48	3.44	0.10	<0.00	0.31
Protein, kg/d	0.56	0.54	0.55	0.56	0.59	0.75	0.81	0.78	0.03	<0.00	0.49
Milk, kg/d	18.4	17.5	17.4	17.3	19.8	23.3	23.8	23.0	1.3	<0.00	0.90
Ruminal pH											
Mean	6.24	6.31	6.30	6.29	6.18	5.63	5.81	5.82	0.06	<0.00	<0.01
Minimum	5.84	5.90	5.87	5.91	5.72	5.02	5.05	5.01	0.07	<0.00	0.89
Maximum	6.57	6.64	6.64	6.61	6.54	6.40	6.59	6.66	0.05	0.49	<0.01
<6.0, min/d	188	100	121	115	321	1,139	885	863	77	<0.00	<0.01
<5.8, min/d	54	24	38	17	93	980	665	712	55	<0.00	<0.00
<5.6, min/d	6	1	8	0	25	760	468	553	53	<0.00	<0.01
Ruminal SCFA, mM											
Total	83.1	82.2	90.6	83.1	87.2	109.9	113.0	100.1	4.41	<0.00	0.22
Acetate	46.0	46.2	57.0	51.2	47.6	54.7	53.8	49.4	2.23	0.91	<0.01
Propionate	18.0	18.2	17.1	16.9	19.7	26.2	32.6	27.1	1.97	<0.00	0.11
Butyrate	11.5	10.7	10.2	9.6	12.2	18.9	17.0	14.9	1.00	<0.00	0.22
Isobutyrate	3.2	2.7	2.5	2.1	3.1	3.2	3.0	3.6	0.37	0.13	0.47
Isovalerate	1.9	1.9	1.6	1.4	2.0	3.4	2.6	2.3	0.21	<0.01	0.12
Valerate	2.5	2.5	2.2	1.8	2.6	3.4	3.9	2.8	0.26	<0.01	0.26
Plasma BHBA, μM	665	758	761	796	683	1,076	1,066	969	79	0.04	0.52

¹All cows ($n = 16$) received HF diet during wk 0 and then were assigned to either the HC ($n = 8$) or HF ($n = 8$) diet during wk 1, 2, and 3.

²Because all cows received the HF diet during wk 0, the effect of treatment was best described by the interaction between diet and time. Helmert contrasts were used to describe the interaction between week 0 and weeks 123 and week 1 and weeks 23.

Table 4. Least squares means of fold change of the expression of genes in rumen tissue from cattle fed the high-concentrate diet (HC) relative to cattle fed the high-forage diet (HF)¹

Gene ²	Gene expression fold change for HC relative to HF				SE	P-value ³
	wk 0	wk 1	wk 2	wk 3		
IGF signaling						
<i>IGFBP3</i>	1.08	0.67*	0.93	0.76*	0.09	<0.00
<i>IGFBP5</i>	1.04	1.66*	1.90*	1.82*	0.24	<0.01
Metabolic						
<i>MCT1</i>	0.95	1.23	1.03	1.06	0.19	0.37
<i>PPARA</i>	0.93	1.08	1.20	0.94	0.13	0.30
<i>HMGCS2</i>	1.01	0.99	1.09	1.01	0.20	0.93
<i>SREBP1</i>	0.91	1.11	1.14	1.03	0.13	0.07
<i>SREBP2</i>	0.88	0.93	0.99	0.98	0.11	0.47
<i>HMGCS1</i>	0.84	0.71*	0.80	0.79	0.13	0.65
<i>FDPS</i>	0.87	0.93	0.85	0.88	0.09	0.83

¹All cows (n = 16) received HF diet during wk 0 and then were assigned to either the HC (n = 8) or HF (n = 8) diet. Gene expression values for HF cattle were normalized to 1 within each week individually.

²*IGFBP* = IGF-binding protein; *MCT1* = monocarboxylic acid transporter 1; *PPARA* = peroxisome proliferator-activated receptor alpha; *HMGCS* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase; *SREBP* = sterol regulatory element-binding protein; *FDPS* = squalene synthase 1.

³Helmert contrasts were used to describe the week effect between week 0 and weeks 123.

*Weekly means for HC are different ($P < 0.05$) from HF (or 1).

The mRNA expression values of both *IGFBP3* and *IGFBP5* were responsive ($P < 0.01$) to treatment. In the case of *IGFBP3*, the mRNA expression was downregulated during wk 1 ($P < 0.01$; 0.67 ± 0.09) and wk 3 ($P < 0.05$; 0.76 ± 0.12) in the HC cattle compared with the HF cattle. Conversely, *IGFBP5* was upregulated in HC cattle relative to HF cattle by 1.66 ± 0.15 fold ($P < 0.05$), 1.90 ± 0.36 fold ($P < 0.01$), and 1.82 ± 0.28 fold ($P < 0.01$) during wk 1, 2, and 3, respectively.

The Helmert contrasts for metabolic gene expression displayed no differences between HC and HF cattle ($P \geq 0.05$). The *HMGCS1* gene was downregulated in HC cattle during wk 1 ($P = 0.02$; 0.72 ± 0.09) and a trend of downregulation was detected during wk 3 ($P = 0.10$; 0.79 ± 0.16). Furthermore, the relative mRNA expression values of *HMGCS1* and *FDPS* (cholesterol biosynthesis genes) in HC cattle were correlated with one another ($R^2 = 0.70$; $P < 0.01$).

DISCUSSION

The goal of this study was to characterize the expression of metabolic and proliferative target genes in the RE of lactating dairy cattle during the transition from a low- to high-energy diet. To achieve our goal, we established a controlled feeding protocol to minimize variation in DMI, which is a common occurrence in cattle fed rapidly fermentable diets ad libitum (Bevans et al., 2005; Gozho et al., 2005; Penner et al., 2009). The subacute form of ruminal acidosis was detected in every HC cow during all treatment weeks. Similar to a previous study in nonlactating dairy cattle (Steele et

al., 2011a), the lowest ruminal pH values were recorded during the first week of the grain challenge. The ruminal pH increased ($P < 0.01$) from wk 1 to 2; however, the cattle still remained in a state of SARA. These results are in agreement with previous studies in lactating dairy cattle reporting that ruminal pH increases during prolonged exposure to a high-grain diet (Matthé et al., 2003; Penner et al., 2007). Despite being in a state of SARA, milk volume and protein production was higher in HC cattle, a typical production response when the energy density of the diet is increased (Lykos et al., 1997; Keunen et al., 2002).

In accordance with ruminal pH results, the HC cattle displayed a significant increase in ruminal SCFA concentration and the ratio of acetate to propionate was decreased, which is in agreement with previous experiments (Harmon et al., 1991; McLeod and Baldwin, 2000). Among measured SCFA, ruminal butyrate in HC cattle displayed the largest proportional increase in concentration (Table 3), a common observation among several experimental models (Blanch et al., 2009; Penner et al., 2009). This is of particular interest, as ruminal butyrate induces proliferation and epithelial growth in the rumen (Sakata and Tamate, 1978; Mentschel et al., 2001; Gorka et al., 2009) and in the large intestine (Hamer et al., 2008). Therefore, it is possible that the increase in ruminal butyrate during a grain challenge may trigger RE adaptation and growth.

A significant portion of SCFA, especially ruminal butyrate, is metabolized by the RE before entering the portal-drained viscera (Bergman, 1990). It is likely that the dramatic increases in ruminal substrate that occur

in a state of SARA may influence the flux of key metabolic pathways in the RE. Ketogenesis is a dominant pathway of the RE (Leighton et al., 1983) and the increase in ruminal substrate can be expected to increase RE ketone body synthesis (Lane et al., 2002). In this study, circulating levels of BHBA at 1600 h (4 h after second grain feeding) were higher in HC cattle relative to HF cattle. Increased concentrations of circulating BHBA have been shown in multiple studies that compare HF and HC diets (Penner et al., 2009; Steele et al., 2009, 2011a,b) and likely reflect a mass action effect of increased substrate for RE ketogenesis.

Gene Expression

To date, all efforts to elucidate the molecular mechanisms orchestrating RE adaptation have focused on comparing mRNA expression of nonlactating ruminants fed an HF to those fed an HC diet. Many of the initial studies failed to uncover changes in mRNA expression in the RE and noted large variability in expression values (Penner et al., 2009). Despite these challenges, several recent studies have successfully identified responsive genes at the mRNA level in nonlactating cattle using microarray technology (Steele et al., 2009, 2011a,b; Taniguchi et al., 2010). Although the expression of mRNA does not necessarily mean that the level of functional protein is expressed in a similar fashion, it is the essential first step when quantifying the molecular response to treatment.

Metabolic Gene Expression

It has been proposed that, in the case of ketogenic gene expression, largely based on the hepatocyte model, the mitochondrial isoform of *HMGCS2* controls RE ketogenesis (Hegardt, 1999). The promoter region of *HMGCS2* contains a peroxisome proliferator-activated receptor response element and its mRNA expression is thereby controlled by *PPARA* transcriptional factor (Meertens et al., 1998). In this study, the relative mRNA expression of *HMGCS2* and *PPARA* were not different between the HF and HC diets. This is in agreement with previous studies that reported no changes in RE ketogenic enzyme gene expression despite elevated levels of circulating BHBA (Penner et al., 2009; Steele et al., 2011b). Harmon et al. (1991) found no differences in the production of ketone bodies in rumen papillae slices from cattle fed a 90% concentrate or 90% forage diet. These results suggest that the changes in RE ketogenesis noted above are dependent upon shifts in available substrate with no changes in the expression of ketogenic enzymes. Furthermore, many metabolic genes are diurnally expressed in the gastrointestinal

tract of mice (Hoogerwerf et al., 2007); therefore, it is plausible that the same occurs in the RE. Based on the daily change in ruminal pH in this study (Figure 1; Table 3), it is possible that the expression of ketogenic genes fluctuates during the day.

It has recently been shown that the mRNA expression of genes involved in cholesterol homeostasis is altered in the RE of nonlactating dairy cattle during ruminal acidosis (Steele et al., 2011b). Intercellular hypercholesterolemia can influence a variety of biological processes, such as cellular permeability, proliferation, and inflammation (Kleemann and Kooistra, 2005); therefore, it is tightly regulated (Simons and Ikonen, 2000). Because SCFA are a precursor of cholesterol, the amount of substrate available for RE cholesterol biosynthesis is increased during a grain challenge and the downregulation of these genes may be a means of maintaining intracellular cholesterol homeostasis. In this study, a downregulation of *HMGCS1* was detected in HC compared with HF cattle only during the first week of the grain challenge when the most severe form of SARA was diagnosed. The expression of *HMGCS1* was correlated with *FDPS*, which are both under the transcriptional control of *SREBP2*. The inactive form of *SREBP2* resides on the endoplasmic reticulum and can translocate to the nucleus after its cleavage (Horton et al., 2002). Because the buildup of cellular cholesterol can block the cleavage of *SREBP2* (Hara et al., 1999; Alvaro et al., 2008), it can be hypothesized that the buildup of sterols in the RE may suppress nuclear translocation of *SREBP2*, thereby downregulating the expression of cholesterol biosynthesis genes.

IGFBP Expression

It has been well established that increasing dietary energy increases RE proliferation (Goodlad, 1981). Among factors triggering these changes, the luminal increase in SCFA and IGF-1 has received much attention in RE research (Penner et al., 2011). By increasing dietary energy, circulating IGF-1 concentration is increased in the ruminant (Shen et al., 2004), and in cell culture, IGF-1 has been shown to increase RE proliferation (Baldwin, 1999). The IGFBP modulate IGF-1 cellular events in a variety of cell lines (Firth and Baxter, 2002) and have been shown to be related to proliferation in bovine mammary epithelial cells (McGuire et al., 1992).

In this study, we found that *IGFBP5* mRNA expression was increased and *IGFBP3* expression was decreased, which is in agreement with our previous findings in nonlactating dairy cattle (Steele et al., 2011a). It has been well characterized that *IGFBP3* opposes IGF-1 events (Albiston et al., 1992); therefore, its

downregulation may encourage cellular growth in the RE. By contrast, *IGFBP5* potentiates IGF-1 events; therefore, its upregulation may promote proliferation. In small and large intestinal cell lines it has been shown that SCFA, in particular butyrate, can modulate the expression of IGFBP (Sanderson, 2004). In a transcriptional study, butyrate induced changes in expression of several IGFBP in kidney epithelial cells (Li and Li, 2006). Furthermore, butyrate downregulates the expression of *IGFBP3* in intestinal epithelial cells, thereby blocking apoptosis (Sanderson, 2004). This finding is in agreement with Mentschel et al. (2001) who found that ruminal butyrate infusions decreased apoptosis in RE. Therefore, it is feasible that differences in IGFBP expression can influence RE growth independent of the level of IGF-1 in circulation during a grain challenge.

CONCLUSIONS

Feeding cattle a diet rich in rapidly fermentable carbohydrates increased milk production and milk protein yield in addition to dramatically altering the rumen fermentation profile. The HC diet caused rumen pH to decline and ruminal SCFA to increase, especially ruminal butyrate. The relative mRNA expression of *IGFBP5* increased, whereas *IGFBP3* and *HMGCS1* were decreased in the RE when cattle were fed a diet high in concentrate. The differential expression of these genes offers new insights into the molecular adaptation of the rumen epithelium to high-grain diets. Enhancing our knowledge of the adaptation of the rumen epithelium to high-grain diets may lead to the development of novel technologies or feeding strategies to mitigate the detrimental effects that SARA imposes on ruminant production systems.

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