Butyrate-Mediated Genomic Changes Involved in Non-Specific Host Defenses, Matrix Remodeling and the Immune Response in the Rumen Epithelium of Cows Afflicted with Subacute Ruminal Acidosis

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ABSTRACT

Subacute Ruminal Acidosis (SARA) is a disorder in cattle which can lead to chronic inflammation in the rumen epithelium, known as rumenitis. Butyrate has been shown to attenuate some of the detrimental effects of inflammatory gastroenteral disorders but the molecular mechanisms mediated by butyrate have not been defined. The objective of this study was to define the inflammatory-related genomic changes responsible for the beneficial effects of butyrate. Experimentally, 16 fistulated dairy cows at mid-lactation were fed a SARA-inducing (45% non-fiber carbohydrate) diet beginning 2 days before the beginning of treatment and continuing throughout the experiment. Cows were then evenly divided into treatment groups where a carrier with (n = 8) or without (n = 8) supplemental butyrate (2.5% initial DM intake) was deposited into the rumen daily for 7 days. The minimum rumen pH was higher in cows with supplemental butyrate (4.96±0.09 to 5.20±0.05, p = 0.040), but mean pH, maximum pH and the duration for which rumen pH was below 5.6 was unaffected. Free plasma Lipopolysaccharide (LPS) concentration was unaffected by treatment as was the concentration of Serum Amyloid A (SAA), although the LPS Binding Protein (LBP) concentration was increased by the addition of butyrate to the rumen (6.91±0.29 to 7.93±0.29 µg mL⁻¹, p = 0.024). Of the rumen Short Chain Fatty Acids (SCFA) tested, only butyrate showed a pronounced treatment effect, rising from 8.60±0.94 to 21.60±0.94 mM (p≤0.0001). Plasma Beta-Hydroxybutyrate (BHBA) concentration also increased (799.50±265.24 to 3261.63±265.24 µM, p<0.001). Butyrate infusion did not affect milk parameters (total fat, lactose, total protein and LOS); however, when related to dry matter intake, milk production efficiency was increased (p = 0.035). Microarray and qRT-PCR analyses of rumen papillae biopsies collected on day 7 found that butyrate administration affected (p≤0.05) the expression of genes involved in Non-Specific Host Defense (NSHD), Remodeling or adaptation (RM) and Immune Response (IR). Of the 49 genes tested by qRT-PCR, 9 (LCN2, MMP1, MUC16, GPX2, CSTA, FUT1, SERPINE2, BCAM, RAC3) were upregulated, 20 (MTOR, AKIRIN2, NFKBIZ, NFKB2, ACVR2A, LAMB1, FRS2, PPARD, LBP, NEDD4L, SGK1, DEDD2, MAP3K8, PARD6B, PLIN2, ADA, HPGD, FMO5, BMP6, TCHH) were downregulated and 20 were unchanged due to butyrate administration in the proximal gastrointestinal tract.

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These results demonstrate the potential protective effect and molecular mechanisms involved in a novel butyrate treatment for inflammatory gastrointestinal conditions.

**Keywords:** Butyrate, Epithelium, SARA, Inflammation, Rumen, Wound Healing, Gene Expression

### 1. INTRODUCTION

SARA is a chronic pathological inflammatory condition of the rumen, affecting approximately 20% of all dairy cattle in North America (Garrett et al., 1999). Although attempts to define the etiology of SARA have gained headway in the past 10 years, its precise mechanisms remain uncertain. Due primarily to the microbial fermentation to SCFA of soluble starch of high grain diets, SARA can lead to liver abscesses, laminitis and sloughing of the rumen epithelium, compromising epithelial integrity (Steele et al., 2011a). Despite the fact that the transmigration of microbes across the rumen wall has been documented in cases of SARA, we have shown that this effect involves the immune system, although the extent of immune system stimulation is limited to local events and is not detectable systemically (Dionissopoulos et al., 2012a; 2012b). Experimentally, we have shown that adaptation to a SARA diet takes place within three weeks (Steele et al., 2011a) and previous experiments have shown that one of the principle methods by which the rumen adapts to an acidic diet is by increasing papillae size and thus the total absorptive surface area of the rumen (Gabel et al., 2002), by increasing cellular turnover and an overall increase in total epithelial cell number (Goodlad, 1981).

There is strong evidence to support the involvement of butyrate in both normal and pathological conditions of the Gastrointestinal Tract (GIT) in both humans and experimental animals (Guilloteau et al., 2010). Roediger (1990), found a direct correlation between the severity of ulcerative colitis and the levels of butyrate. Since butyrate has been previously shown to be an important energy source for the GIT (Ahmad et al., 2000), we can speculate that low butyrate levels likely lead to an energy-deficient state (low ATP) and low metabolic rate in the rumen. These effects may culminate in the promotion of apoptosis and hence the degradation of the epithelial barrier. Indeed, butyrate can directly stimulate epithelial cell proliferation and differentiation (Aoyama et al., 2010; Zhang et al., 2010), both events which are hallmarks of the healing and restoration of normal function following an immunological insult. The ability to reduce or modulate the severity of the immune response is critical to our understanding of how sub-acute inflammatory conditions such as SARA exert their negative effects. A reduction in the duration and the extent of the immune response can have beneficial effects since nutrient partitioning favouring immune cell recruitment and growth can be limited (Dionissopoulos et al., 2006). In addition, reductions in the magnitude and severity of the immune response have been shown to favour wound healing and remodelling following injury (Eming et al., 2007). Since the reduction of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α can reduce the length and severity of the immune response (Johnson, 1997), it follows that limiting such a response with naturally occurring substances in sub-clinical disease can have beneficial effects.

An understanding of the molecular mechanisms involved in rumen epithelial remodelling in sub-clinical disease would be incomplete without an exploration of inflammatory events at the gene expression level. The question that invariably arises centers around the principle effectors or transcription factors that mediate the immune cascade, which are known to be nuclear factor κB (NF-κB/p50/p65) and Peroxisome Proliferator Activated Receptor types (PPARs) in such a setting (Hoffmann and Baltimore, 2006; Calder, 2008). NF-κB, originally studied as a promoter of lymphocyte maturation (Kumar et al., 2004), has been found to have a much broader scope of action. NF-κB is found to be present in promoter sequences of pro-inflammatory cytokines, the inflammatory enzyme COX-2 and in leukocyte adhesion molecules (Sigal, 2006; Perkins 2007). Because of its importance, it can be seen how NF-κB and its cofactors will likely be targets for future immunomodulatory agents such as butyrate (Jobin and Sartor, 2000a; 2000b; Kinoshita et al., 2002; Zhang et al., 2006). Briefly, stimulation by bacterial or viral antigens, cytokines and Reactive Oxygen Species (ROS), IKK (an NF-κB kinase) phosphorylates the inhibitory subunit of the NF-κB complex, IκBα, prompting its dissociation from the p50/p65 NF-κB complex. Once free of its inhibitory complex, NF-κB traverses the nuclear envelope and begins the inflammatory cascade (Hoffmann and Baltimore, 2006).

PPARs are also important transcription factors in gastrointestinal inflammation. However, their effects are thought to act in an opposing manner to that of NF-κB as anti-inflammatory agents (Guri et al., 2010). Most evidence of the anti-inflammatory effect of PPARs comes from studies where subjects with advanced inflammatory bowel diseases generally show decreased levels of PPAR-γ mRNA expression (Desreumaux et al., 1999). More strikingly, in experimental mouse models, treatment with a PPAR-γ agonist reduced symptoms of inflammation.
cortex (Desreumaux et al., 2001). Moreover, butyrate has been shown to stimulate the production of PPAR-γ in cell lines (Kinoshita et al., 2002). Perhaps most importantly is the suggestion that PPAR-γ inhibits NF-κB activation by interfering with the phosphorylation of IκB, preventing its degradation and the subsequent translocation of the NF-κB complex to the nucleus (Ross et al., 1999; Haegeman, 2003; Berghe et al., 2003).

We hypothesize that the addition of exogenous butyrate in cows afflicted with SARA will lessen the extent of immune system stimulation, will help the rumen adapt to a high grain/high energy diet and through adaptation, support changes to an epithelial barrier that is more resistant to insult or injury by pathogenic organisms to meet new metabolic demands. The goal of the experimentation described in this article is to characterize the mechanisms involved in rumen epithelial remodelling and, through butyrate supplementation, lessen the severity and extent of the negative effects of SARA. Here we outline the data relating the Immune Response (IR), rumen epithelial Remodelling (RM) and Non-Specific Host Defence (NSHD) in SARA cows supplemented with butyrate.

2. MATERIALS AND METHODS

2.1. Animals, Treatments and Diet

Sixteen mid-lactation, rumen cannulated (Duffield, 1999) primiparous and multiparous Holstein cows on a mid-lactation Total Mixed Ration (TMR) diet were selected and blocked by Days In Milk (DIM). Prior to the experiment, total Dry Matter Intake (DMI) and milk production were recorded for 7 days. Two days before the start of the study, a concentrate mix was added to the TMR to increase the Non-Fiber Carbohydrate (NFC) to 45.0% in 2 equal increments. According to previously published results, a NFC of 45% would be sufficient to induce SARA in this study (Steele et al., 2012). Each increment was 50% of the total amount of concentrate needed to increase the NFC to 45.0%. The composition of the diet is presented in Table 1.

At the start of the 7-day experiment, each cow was randomly assigned to one of two treatments. Butyrate cows (n = 8) received a ruminal dosing of butyrate (Proformix™, Probiotech Inc., Saint-Hyacinthe, QC) while Control cows received no supplemental butyrate but did receive carrier. The butyrate powder (50% butyric acid, sodium bicarbonate, lime) was dosed at a rate of 2.5% of pre-trial DMI, at 10.00 and 13.30 h daily, to coincide with regular daily feeding times.

### Table 1. Diet fed to lactating Holstein dairy cows with or without supplemental butyrate

| Ingredient                        | %DM |
|-----------------------------------|-----|
| Corn silage                       | 22.9|
| Haylage, 1st cut                  | 22.9|
| Straw                             | 5.1 |
| High moisture corn                | 16.7|
| Protein supplement                | 15.8|
| Grain supplement                  | 16.4|
| Barley grain                      | 60.0|
| Corn grain                        | 20.0|
| Wheat grain                       | 20.0|

**Formulated (Calculated) Analysis**

- DM, %: 52.6
- CP, % of DM: 15.9
- EE, % of DM: 3.4
- Starch, % of DM: 24.5
- NDF, % of DM: 33.7
- Forage NDF, %NDF: 75.2
- NFC, % of DM: 44.0

2.2. Rumen SCFA, LPS and pH

Rumen fluid was collected approximately 3 h after the afternoon feeding from the ventral sac and squeezed through 4 layers of cheesecloth and frozen for analysis of total SCFA by gas chromatography (Steele et al., 2012). Another sample of rumen fluid was analyzed fresh for total free LPS using the chromogenic Limulus amoebocyte lysate end-point assay (Lonza Group LTD., Basel, Switzerland) as previously described (Gozho et al., 2005; Dionissopoulos, 2012b). Rumen pH was recorded continuously for the last two days of the trial using a pH recording system and protocols established by our laboratory (AlZahal et al., 2007).

2.3. Plasma BHBA, LBP and SAA

On days 1 and 7, blood was sampled through the tailhead at 16.30 h. After collection, the blood was stored on ice and subsequently spun at 3000 x g, then stored at -20°C until further processing. Plasma LBP was assayed according to established protocols (Khafipour et al., 2009b). Plasma BHBA was determined by the Animal Health Laboratory (AHL, University of Guelph, Guelph, Ontario) using the method of Williamson et al. (1962). Serum Amyloid A (SAA) protein levels were determined by enzyme-linked immuno assay using a multi-species SAA kit (TriDelta Development, Ltd, Maynooth, County Kildare, Ireland).

2.4. Dry Matter Intake (DMI), Daily Milk Production and Milk Component Analysis

DMI and milk production was calculated daily for each cow on the study. All cows were milked at 05.30 h
and 16.00h daily and their milk production numbers were pooled to establish a total daily milk production value. Milk protein and fat were determined spectroscopically by the CanWest DHI Laboratory (Guelph, Ontario). These values were compiled to yield an average weekly DMI, production value and milk component analysis.

2.5. Rumen Papillae Biopsies for Microarray and qRT-PCR

Rumen papillae were harvested from the rumen ventral sac at the end of experimental day 7 (Steele et al., 2012). Briefly, the rumen contents were partially evacuated to help access and retraction of the rumen ventral sac.

Table 2. Primer design for qRT-PCR analysis. Primer efficiency was calculated using the formula $E = -1 + 10(-1/slope) \times 100$; the slope was derived from the PCR 5-point standard curve ($R^2 \geq 0.99$)

| Accession # | Gene Name | 5'→3' | Efficiency (%) |
|-------------|-----------|-------|----------------|
| NM_174227   | ACVR2A    | F     | 86             |
|             |           | R     | 86             |
| NM_173887   | ADA       | F     | 88             |
|             |           | R     | 88             |
| NM_001110087| AKIRIN2   | F     | 82             |
|             |           | R     | 82             |
| NM_174741   | BCAM      | F     | 94             |
|             |           | R     | 94             |
| XM_002697620| BMP6      | F     | 91             |
|             |           | R     | 91             |
| NM_174008   | CD14      | F     | 85             |
|             |           | R     | 85             |
| NM_001166511| COL4A1    | F     | 94             |
|             |           | R     | 94             |
| NM_001167824| CSTA      | F     | 81             |
|             |           | R     | 81             |
| NM_001076017| DEDD2     | F     | 54             |
|             |           | R     | 54             |
| NM_001193131| FGF7      | F     | 98             |
|             |           | R     | 98             |
| NM_001101304| FMO5      | F     | 84             |
|             |           | R     | 84             |
| NM_001163778| FN1       | F     | 100            |
|             |           | R     | 100            |
| NM_001203000| IGFBP7    | F     | 100            |
|             |           | R     | 100            |
| ENSBTAT0000002549| FRS2 | F     | 93             |
|             |           | R     | 93             |
| NM_177499   | FUT1      | F     | 104            |
|             |           | R     | 104            |
| NM_001163139| GPX2      | F     | 74             |
|             |           | R     | 74             |
| NM_001034419| HPGD      | F     | 88             |
|             |           | R     | 88             |
| NM_001102300| IGFBP7    | F     | 100            |
|             |           | R     | 100            |
| NM_174088   | IL10      | F     | 100            |
|             |           | R     | 100            |
| XM_002691608| LAMB1     | F     | 100            |
|             |           | R     | 100            |
| NM_001038674| LBP       | F     | 95             |
|             |           | R     | 95             |
| XM_002691670| LCN2      | F     | 83             |
|             |           | R     | 83             |
| NM_001046517| LY96      | F     | 86             |
|             |           | R     | 86             |
| NM_001099071| MAP3K8    | F     | 91             |
|             |           | R     | 91             |
| Accession | Gene | Forward | Reverse |
|-----------|------|---------|---------|
| NM_001033608 | MIF | GGAATCTACATCAACTTCTGCGACAT | GAAAGTTGGAGCCGTTCACCA |
| NM_001035411 | MLST8 | AGCCAATCTCCAGGTGAAA | GGCGTACGATGTGATTTG |
| NM_174112 | MMP1 | GACGTCAGCCGTTGTTCTC | TCCTGAGGTCAGTTGCTTC |
| ENSBTAT00000020386 | MTOR | CATTGACGAGTATGTTGAGA | AGAGCATAATCCTGGAAG |
| FN600737 | MUC16 | TGGAACGATAAACGCTCCTA | GGTGGTATGTTGGAGTTG |
| ENSBTAT00000018334 | NEDD4L | GCTGATGGACGCCGAGAA | GGAACGTCCCGTGACAAACTG |
| _NM_001076409 | NFKB1 | CGAATGACAGATGCCTGTATACG | CTGCAAATAGGCAAGATCAGG |
| _NM_001102101 | NFKB2 | CCAGGAACCTGAAGAAGGT | CCAGAGGAATATAGGTGAACT |
| _NM_174726 | NFKBIZ | TGGACTTGGAGGCAACTAATG | TATGCGCAAGACTGACAGT |
| _NM_001098104 | PARD6B | CGCAAGTGAGCTCAGTTTCG | CTTGCGTATGGAGGGAAG |
| _NM_001017953 | PDGFB | ATGGTGTCAGAAAAGATGAAA | GCAGAAGAGGTGGTGATAGG |
| _NM_173980 | PLIN2 | GTGGTGCAAGCCTGTACAG | GCAAGAACTGAGCCCATG |
| _NM_001083636 | PPARD | AATTGTGAGCGGATCTGAAA | GCGCAATGTACTGGCAGTC |
| _NM_001105323 | PTGS1 | CCCCAAATGAGACCCTGGAT | CCAAAATGAGACCCCTGGAT |
| _NM_174445 | PTGS2 | AATCTTCAGTCAGTCAAG | TTAGGCACTGATGTTGATG |
| _NM_001099179 | RAC3 | GCAATGATGTAGTTGAGG | GGAAGACGATAGCAAAG |
| _NM_174669 | SERPINE2 | CCCCCAGTTGGCTATTTG | TGGATTTGCTCCGGTGGTA |
| _NM_001102033 | SGK1 | TTCTCTGGAAGACACAA | AACATCGTGCTCCGGACATA |
| _NM_001076223 | SMAD1 | ATTGGAAATGCTGCGAGTT | GCTGTCGAGGATTGTAT |
| ENSBTAT0000000311 | STAT6 | GAGCCCTGATGGAACTCT | AAGTTGAGCAGAATGAG |
| BC151676 | TCHH | ATGCTCTGATGTGCTTAC | AATCTGAATGAGATAAG |
| _NM_001035313 | TGFBI1 | ATGTCTCCAAGGGTATACAA | TCGTGTCAGACGAA |
| _NM_174198 | TLR4 | GTAAGAAGACGAAACTCA | CAAGAAAGTACAGGTTGAG |
| _NM_001101306 | TNFRSF6B | GCACCCCTTCTCTGGATTATTACATCA | TCCGAAGCCCTCCTGGT|

**Table 2. Continued**
Approximately 150 mg of papillae was cut from the rumen and washed 20 times in ice-cold PBS, then placed in a stabilization agent (RNALater™, Qiagen, Hilden, GmbH) until the RNA could be isolated. Total RNA was isolated as previously described by Steele et al. (2012) using an RNaseasy midi kit (Qiagen, Mississauga, Ontario, Canada). The concentration of RNA was determined using a NanoDrop (ND-1000, NanoDrop Technologies, Wilmington DE). To enhance the purity of the RNA, it was treated with DNase (Invitrogen, Burlington, Ontario, Canada) and the RNA 6000 Nano kit (Caliper Life Sciences, Mountain View, CA). The RNA was then prepared for either microarray analysis, to determine the global expression pattern of genes, or for qRT-PCR, to confirm the relative expression of any genes (Steele et al., 2011b) involved in epithelial RM, the IR, or the NSHD. Where possible, primers were designed to span exon-exon junctions using NCBI/PrimerBLAST Primer Express (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and were verified as specific to the bovine genome using GenBank (National Center for Biotechnology Information, Bethesda, MD). All primer amplicons were confirmed by BLASTN in NCBI, as well as through the generation of dissociation curves to support the fidelity of single product amplification (Steele et al., 2011a), using GAPDH as the housekeeping gene. The stability of GAPDH amplicons were confirmed by low variance (mean Ct = 20.85; variance = 0.10). Primer information including sequences can be found in Table 2. Any treatment imposed differences in mRNA expression was determined using the inverse of qPCR efficiency raised to Delta Ct (Pfalz et al., 2004). The expression values of the Control cows were pooled and expression differences were determined through the comparison of individual Butyrate group values to this index as described previously using a Student’s t-test (Xue et al., 2010). Finally, all differentially expressed genes were subjected to analysis with the Ingenuity Pathway Analysis (IPA) tool (Ingenuity, Inc., Redwood City, CA) in cooperation with the University of Kentucky (Lexington, KY). The methodologies employed herein have been previously published (Steele et al., 2011b). It is important to note however that to determine the significance of differentially expressed genes in this study, the data derived from the microarray analysis was subjected to a pre-screen of 95% confidence and a false discovery rate of 0.1 according to the methods of Benjamini (Reiner et al., 2003).

2.6. Statistical Analysis

Measurements unrelated to the microarray or qRT-PCR analysis were analyzed using the MIXED procedure of SAS (2004) using a previously described model (Steele et al., 2012):

\[ Y_{ij} = \mu + D_i + T_j + (D \times T)_{ij} + \epsilon_{ij}, \]

where, \( Y_{ij} \) is the dependent variable, \( \mu \) is the variable mean, \( D_i \) denotes the fixed effect of diet (\( i = 1, 2 \)), \( T_j \) is the fixed effect of time or day (\( j = 1,.., 7 \)), \( (D \times T)_{ij} \) is the diet by time interaction (\( ij = 1,...,14 \)) and \( \epsilon_{ij} \) represents the random residual error. Both diet and time were analyzed as fixed effects and day was analyzed as a repeated measurement with cow as the subject. The subject cow was treated to covariance structure measurements; the covariance structure resulting in the smallest Bayesian information criterion was used in this analysis.

3. RESULTS

3.1. Physiological Parameters

Table 1 represents the analysis of the diet which was formulated for this study. Both the Control and Butyrate groups received the same diet with the exception of supplemental butyrate in the treatment group.

Total rumen SCFA were analyzed for all animals and the data is presented in Table 3. Total SCFA levels differed significantly only on day 1 of the study and were higher in the Butyrate group (92.76 Vs 78.87 mM ±4.51; \( p = 0.04 \)). However, as expected, total volatile butyrate levels were dramatically higher in the Butyrate group both on day 1 (22.60 Vs 9.88 mM ±0.94, \( p =0.0001 \)) and day 7 (21.60 Vs 8.60 mM ±0.94, \( p =0.0001 \)).

Total plasma LBP and free rumen LPS levels are also presented in Table 3. Plasma LBP levels were significantly higher in the Butyrate vs. the Control group on day 7 of the study (7.93 Vs 6.91 µg mL\(^{-1}\) ± 0.29; \( P = 0.024 \)). Free LPS did not differ significantly for either treatment day between groups.

Also as expected, plasma BHBA levels were increased significantly due to treatment (Table 3). On day 1, Control Vs Butyrate levels were 909.50 vs. 4201.13 µM ±265.24 (\( p=0.001 \)) and on day 7 of the study, levels of BHBA rose significantly from 799.50 to 3261.63 µM ±265.24 (\( p=0.001 \)). Data for the serum acute phase protein SAA is presented in Table 3. No treatment differences were seen (\( p>0.05 \)).

![](image_file)
Table 3. Rumen SCFA, plasma BHBA, plasma LBP, rumen LPS, and SAA concentrations with (Butyrate group) or without (Control group) 2.5% butyrate, DMI. Values are expressed as means ± SEM; n = 8 per Control or Butyrate group. P values represent comparison between treatments on individual days

|                      | Day 1     | Day 7     | SEM  | P value  |
|----------------------|-----------|-----------|------|----------|
|                      | Control   | Butyrate  |      |          |
| Total SCFA (mM)      | 78.87     | 92.76     | 4.51 | 0.0400   |
|                      | 81.82     | 87.59     | 4.51 | 0.3700   |
| Acetate              | 38.20     | 40.40     | 1.90 | 0.4100   |
|                      | 39.20     | 36.40     | 1.90 | 0.3100   |
| Propionate           | 25.80     | 23.90     | 2.70 | 0.6200   |
|                      | 27.70     | 24.40     | 2.70 | 0.4000   |
| Isobutyrate          | 1.70      | 2.20      | 0.29 | 0.2300   |
|                      | 2.50      | 1.90      | 0.29 | 0.1800   |
| Butyrate             | 9.88      | 22.60     | 0.94 | ≤0.0001  |
|                      | 8.60      | 21.60     | 0.94 | ≤0.0001  |
| Isovalerate          | 1.02      | 1.21      | 0.08 | 0.1100   |
|                      | 1.12      | 1.06      | 0.08 | 0.6200   |
| Valerate             | 2.27      | 2.51      | 0.24 | 0.5000   |
|                      | 2.67      | 2.27      | 0.24 | 0.2600   |
| BHBA (µM)            | 909.50    | 4201.13   | 265.24 | ≤0.0010  |
|                      | 799.50    | 3261.63   | 265.24 | ≤0.0010  |
| LBP (µg/mL)          | 6.68      | 6.80      | 0.29 | 0.7660   |
|                      | 6.91      | 7.93      | 0.29 | 0.0240   |
| LPS (EU/mL)          | 52723.00  | 28892.00  | 8485.84 | 0.0570   |
|                      | 14425.00  | 7517.25   | 8485.84 | 0.5700   |
| SAA (ng/mL)          | 305.12    | 322.28    | 27.87 | 0.6700   |
|                      | 358.41    | 384.55    | 27.87 | 0.5100   |

Table 4. Effect of butyrate on rumen pH in Holstein cows with (Butyrate group) or without (Control group) butyrate supplementation at 2.5% DMI. Time in min/day; AUC = area under the curve in pH ≤5.6× min/day

| Treatment | Butyrate | Control | P     |
|-----------|----------|---------|-------|
| Min       | 5.20±0.05| 4.96±0.09| 0.04  |
| Mean      | 5.67±0.04| 5.66±0.06| 0.89  |
| Max       | 6.36±0.11| 6.55±0.16| 0.27  |
| Time pH ≤ 5.6 | 536±89 | 598±97 | 0.65  |
| AUC       | 87±26    | 168±40  | 0.11  |

Table 5. Dry matter intake, daily milk production, and daily milk production ratio. *The milk production ratio is adjusted for variations in dry matter intake related to treatment effects and indicates that the butyrate group was more efficient in the production of milk than was the control group

|                      | Control   | Butyrate  | SEM  | P-value  |
|----------------------|-----------|-----------|------|----------|
| Dry Matter Intake, DMI (kg/day) | 24.40     | 17.41     | 0.77 | ≤0.0001  |
| Daily Milk, DMP (kg/day)          | 41.93     | 34.74     | 2.50 | 0.0617   |
| Daily Milk Production (kg/day)    | 1.67      | 1.96      | 0.09 | 0.0354   |
| Ratio, MPR (DMP/DMI)*             | 0.355     | 0.355     |      |          |

As indicated previously, the effect of butyrate supplementation was examined on pH parameters in the rumen (Table 4). The minimum rumen pH reached during the time course of this study was lower in the Control group than the Butyrate group (4.96±0.09 Vs 5.20±0.05; p = 0.04). However, the addition of exogenous butyrate had no effect on the mean pH, max pH, the duration at which pH was below 5.6, or the area under the pH curve (p>0.05). DMI, milk production and production efficiency are presented in Table 5. Addition of exogenous dietary butyrate significantly lowered the DMI of the animals (17.41 Vs 24.40 kg day⁻¹ ±0.77; p<0.0001). Daily milk production was unaffected by treatment: 41.93 kg day⁻¹ ±2.50 in the Control group to 34.74 kg day⁻¹ ±2.50 in the Butyrate group (p>0.05). Interestingly, the daily milk production ratio, defined as the daily milk production Vs DMI, was significantly higher in the Butyrate group than in the Control group (1.96 Vs 1.67±0.09; p = 0.035).

3.2. Microarray Screen, qRT-PCR and Pathway Analysis

Gene expression results along with fold changes are presented in Table 6. Microarray data indicated the significant differential expression of 1191 genes (data not shown). These genes were screened in turn for involvement in NSHD, RM and IR pathways. Of the 49 genes selected for further analysis, 29 were confirmed significantly differentially expressed by qRT-PCR. Nine genes were found to be upregulated, 20 were downregulated and 20 were unaffected by butyrate supplementation. In turn, these genes were analyzed and placed into context-specific NSHD, RM and IR pathways, Fig. 1 and Table 7.
| Symbol         | Entrez Gene Name                      | Fold change | P-value |
|---------------|---------------------------------------|-------------|---------|
| LCN2          | lipocalin 2                            | 27.0        | 0.000   |
| MMP1          | matrix metalloproteinase 1             | 21.6        | 0.002   |
| MUC16         | mucin 16, cell surface associated      | 14.9        | 0.001   |
| GPX2          | glutathione peroxidase 2 (gastrointestinal) | 10.4       | 0.000   |
| CSTA          | cystatin A (stefin A)                  | 7.1         | 0.000   |
| FUT1          | fucosyltransferase 1                  | 4.3         | 0.000   |
| SERPINE2      | serpin peptidase inhibitor, clade E member 2 | 3.7       | 0.000   |
| BCAM          | basal cell adhesion molecule          | 1.5         | 0.006   |
| RAC3          | ras-related C3 botulinum toxin substrate 3 | 1.4       | 0.044   |
| MTOR          | mechanistic target of rapamycin       | -1.4        | 0.027   |
| AKIRIN2       | akirin 2                              | -1.7        | 0.000   |
| NFKB1Z        | nuclear factor of kappa light polypeptide gene enhancer inhibitor zeta | -1.7 | 0.001 |
| ACVR2A        | activin A receptor, type IIA           | -1.7        | 0.001   |
| LAMB1         | laminin, beta 1                       | -1.7        | 0.020   |
| FRS2          | fibroblast growth factor receptor substrate 2 | -1.8 | 0.001 |
| PPARD         | peroxisome proliferator-activated receptor delta | -1.8 | 0.000 |
| NFKB2         | nuclear factor of kappa b light polypeptide gene enhancer 2 | -2.0 | 0.001 |
| LBP           | lipopolysaccharide binding protein     | -2.0        | 0.006   |
| NEDD4L        | E3 ubiquitin protein ligase            | -2.1        | 0.000   |
| SGK1          | serum/glucocorticoid regulated kinase 1 | -2.3 | 0.001 |
| DEDD2         | death effector domain containing 2     | -2.6        | 0.000   |
| MAP3K8        | mitogen-activated protein kinase kinase 8 | -2.6 | 0.001 |
| PARD6B        | par-6 partitioning defective 6 homolog beta | -2.6 | 0.000 |
| PLIN2         | perilipin 2                           | -2.9        | 0.000   |
| ADA           | adenosine deaminase                   | -3.3        | 0.000   |
| HPGD          | hydroxyprostaglandin dehydrogenase 15-(NAD) | -3.3 | 0.000 |
| FMO5          | flavin containing monoxygenase 5       | -3.4        | 0.000   |
| BMP6          | bone morphogenetic protein 6           | -3.7        | 0.000   |
| TCHH          | Trichohyalin                          | -5.0        | 0.000   |
| CD14/TLR4/LY96| TLR4 Receptor Complex                  | unchanged   | ns      |
| EGFR          | epidermal growth factor                | unchanged   | ns      |
| EGFR          | epidermal growth factor receptor       | unchanged   | ns      |
| ERK1/2        | extracellular signal related kinase 1/2 | unchanged | ns      |
| Fgf           | fibroblast growth factor               | unchanged   | ns      |
| Fgfr          | fibroblast growth factor receptor      | unchanged   | ns      |
| FN1           | fibronectin 1                          | unchanged   | ns      |
| IGFBP7        | IGF binding protein 7                  | unchanged   | ns      |
| IL10          | interleukin 10                         | unchanged   | ns      |
| LY96          | lymphocyte antigen 96                  | unchanged   | ns      |
| MIF           | macrophage migration inhibitory factor | unchanged   | ns      |
| MLST8         | MTOR associated protein                | unchanged   | ns      |
| NFKB1         | nuclear factor of kappa light polypeptide gene enhancer 1 | unchanged | ns |
| PTGS1         | prostaglandin-endoperoxide synthase 1  | unchanged   | ns      |
| PTGS2         | prostaglandin-endoperoxide synthase 2  | unchanged   | ns      |
| SMAD1         | mothers against decapentaplegic homolog 1 | unchanged | ns     |
| STAT6         | signal transducer and activator of transcription 6, interleukin-4 induced | unchanged | ns |
| TGFBI11       | transforming growth factor beta-1-induced transcript 1 | unchanged | ns |
| TLR4          | toll-like receptor 4                   | unchanged   | ns      |
| TNFRSF6B      | Tumor necrosis factor receptor superfamily member 6B | unchanged | ns    |
Table 7. Gene expression results and interpretation. *Biological interpretation in lieu of experimental model. Abbreviations: NSHD, non-specific host defense; Remodel, remodeling of cellular architecture; Other, non-categorized; IR, immune response; ECM, extracellular matrix; ROS, reactive oxygen species; PG, prostaglandins; NF-κB, nuclear factor kappa B; TGF-β, transforming growth factor type beta; TLR4, toll-like receptor 4

| Category | Symbol | Function | Fold Change | Contextual Meaning* | Ref. |
|----------|--------|----------|-------------|---------------------|------|
| NSHD     | LCN2   | Iron sequestration from bacteria | 27 | ↓ bacterial growth | (Flo et al., 2004; Schmidt-Ott et al., 2007) |
| RM       | MMP1   | ECM remodeling; degradation of ECM | 21.6 | Adaptation | (Pendas et al., 1996) |
| NSHD     | MUC16  | Mucous production and protection of epithelium | 14.9 | ↓ bacterial attachment | (Perez and Gipson, 2008) |
| NSHD     | GPX2   | Protection against bacterial production of ROS in rumen | 10.4 | ↓ bacterial attachment | (Brigelius-Flohe and Kipp, 2012) |
| NSHD     | CSTA   | Promotes cell-cell adhesion | 7.1 | ↓ bacterial pathogenicity | (Blaydon et al., 2011) |
| NSHD     | FUT1   | Regulates pathogen attachment to epithelium | 4.3 | ↓ bacterial attachment | (Yan et al., 2003; Wang et al., 2012) |
| NSHD     | SERPINE2 | Serine protease providing resistance to bacterial colonization | 3.7 | ↓ bacterial attachment | (Luo et al., 2011) |
| RM       | BCAM   | ECM receptor | 1.5 | Adaptation | (Eyler and Telen, 2006) |
| RM       | RAC3   | Cell growth, reorganization of the cytoskeleton | 1.4 | Adaptation | (Haataja et al., 2002) |
| RM       | MTOR   | Various; reorganization of the cytoskeleton | -1.4 | Adaptation | (Sarbassov et al., 2004) |
| IR       | AKIRIN2 | Downstream TLR4 effector; regulates NF-κB | -1.7 | Adaptation ↓IR | (Goto et al., 2008) |
| IR       | NFKBIZ | NF-κB regulator | -1.7 | Adaptation ↓IR | (Cowland et al., 2006; Totzek et al., 2006) |
| RM       | ACVR2A | Reorganization of the cytoskeleton; downregulates immune response | -1.7 | Adaptation ↓IR | (Lebrun et al., 1999; Tsuichida et al., 2004) |
| RM       | LAMB1  | ECM protein necessary for signaling and structural integrity | -1.7 | Adaptation | (Taniguchi et al., 2009) |
| RM       | FRS2   | ECM reorganization; FGF receptor signaling | -1.8 | Adaptation | (Ong et al., 2000) |
| RM       | PPARD  | ECM reorganization | -1.8 | Adaptation | (Tan et al., 2007) |
| IR       | NFKB2  | NF-κB regulator | -2.0 | Adaptation ↓IR | (Al-Sadi et al., 2010) |
| IR       | LBP    | Mediator of LPS activity | -2.0 | Adaptation ↓IR | (Gray et al., 1993) |
| IR       | NEDD4L | Negative regulator of TGF-β signaling | -2.1 | Adaptation ↓IR | (Kuratomi et al., 2005) |
| Other    | SGK1   | Na⁺ channel regulator | -2.3 | Unknown | (Grahammer et al., 2006) |
| RM       | DEDD2  | ECM/cellular remodeling; low levels decrease apoptosis | -2.6 | Adaptation | (Lee et al., 2002) |
| IR       | MAP3K8 | Various; low levels decrease immune response | -2.6 | Adaptation ↓IR | (Miyoshi et al., 1991; Hatziapostolou et al., 2011) |
| NSHD     | PARD6B | Cell polarization; low levels may increase tight junction integrity | -2.6 | Adaptation | (Yamanaka et al., 2003) |
| IR       | PLIN2  | Maintenance of adipose tissue; may decrease macrophage recruitment | -2.9 | Unknown ↓IR | (Hao et al., 2011) |
| NSHD     | ADA    | Maintenance of the immune system | -3.3 | Adaptation ↓IR | (Wilson et al., 1991) |
| NSHD     | HPGD   | Metabolism of PG; low levels maintain protective PG | -3.3 | Adaptation | (Cho et al., 2006) |
| Other    | FMO5   | Metabolism of drugs, pesticides, xenobiotics | -3.4 | Unknown | (Jannmohamed et al., 2001) |
| RM       | BMP6   | Matrix growth factor; low levels may aid reepithelialization | -3.7 | Adaptation | (Kaiser et al., 1998) |
| NSHD     | TCHH   | Keratin filament-associated protein; low levels prevent rigidity of ECM | -5.0 | Adaptation | (Steinert et al., 2003) |
4. DISCUSSION

It has been shown that adaptation to energy-dense, high carbohydrate diets in dairy cows is facilitated by structural changes in the rumen favouring extracellular matrix remodelling (Steele et al., 2011a). This effect does not always follow a profound inflammatory phase and it was determined that favourable adaptive changes in the rumen take place beginning 1 week after a high grain challenge. The aim of the current study was to determine the efficacy of butyrate supplementation in ameliorating the negative ruminal effects induced by feeding a high carbohydrate to lactating dairy cows. In addition, since the effects of SARA on rumen physiological dynamics have been reported (Keunen et al., 2002; Penner et al., 2007), we sought to determine the effects of butyrate treatment on milk production, milk components, pH, SCFA, SCFA metabolism, the effects on NSHD and evidence for an IR.

4.1 Physiological Parameters

We previously reported pH results in cows fed a high grain diet that is in agreement with the current study (Steele et al., 2012). Of note however, is the effect of supplemental butyrate on minimum pH reported; the rumen of cows in the Control group reached a lower pH than did the Butyrate group. Since butyrate itself is a weak acid, it is unlikely that buffering by its conjugate base would be responsible for this effect. However, it is possible that butyrate altered the metabolic flux of the rumen, favouring the neutralization of $H^+$ through the efflux of bicarbonate. Similar effects have been reported (Kristensen et al., 1998; Penner et al., 2009a; Aschenbach et al., 2010).

A high dietary carbohydrate load such as that seen in this study is expected to favour ketogenesis. In the rumen, SCFA are created as the result of fermentation by resident microbes and are metabolized within the site of absorption in the epithelia of the rumen, prior to making their appearance as substrates in the general tissue beds (Bergman, 1990; Kristensen et al., 1998). The ensuing hyperketonemia and ketosis can be confirmed through plasma analysis of BHBA levels (Penner et al., 2009b). Indeed, plasma BHBA levels were raised considerably in the Butyrate group as expected, which confirmed experimental ketosis. This had a direct effect on DMI, which was substantially lower in the Butyrate group; the effects of high SCFA levels on increasing satiety and decreasing appetite have been documented previously (Arora et al., 2011; Lin et al., 2012). And although supplemental butyrate had no effect on daily milk production, milk fat, or milk protein, the efficiency of milk production was higher in the Butyrate cows. These results appear to be a function of a decreased DMI. The fact that the milk parameters differed in grain content from previous studies is likely due to the lower energy density of the current SARA diet (Lykos et al., 1997; Keunen et al., 2002).

In such a carbohydrate-rich diet, both commensal and pathogenic microbes thrive. This is because such microbes can use the more readily available sugars as a preferential metabolic substrate (Motoi et al., 1993; Gozho et al., 2005; Thibault et al., 2010). Although the grain level in our study was not as high as that previously reported, the 45% NFC is sufficient to promote the growth of gram negative bacteria, which is correlated with the levels of rumen LPS (Zebeli and Ametaj, 2009). Although the LPS levels in this study are in relative agreement with those published earlier (Khafipour et al., 2009a; 2009b; Li et al., 2012), it is not clear whether they are in sufficient concentration to favour transmigration and to trigger an immune response. In order for such a response to exist, free LPS must be transported to effector cells of the immune system by the soluble acute phase protein, LBP (Muta and Takeshige, 2001). Here we report that butyrate supplementation increases plasma levels of LBP. However, this effect may be muted by the addition of butyrate, as several groups have reported reductions in the downstream effects of LPS-mediated induction of the immune system (Chakravortty et al., 2000; Huuskonen et al., 2004; Morikawa et al., 2004; Ni et al., 2010) under similar conditions. In addition, SAA, also an acute phase protein, was unaffected by the addition of butyrate. Together, the data for LPS, LBP and SAA contradict earlier findings using similar models (Dionissopoulos et al., 2012a; 2012b), that although SARA can have profound effects on the physiology of the rumen, the bacterial overgrowth that typically occurs is not sufficient enough to result in a systemic inflammatory response. The tissue signals generated as a result of SARA at the site of tissue damage (Steele et al., 2011a) likely act locally in an autocrine or paracrine manner.

4.2. Gene Expression and Pathway Analysis

The rumen epithelium is part of a remarkable system that is meant to facilitate nutrient absorption and to function as a barrier to disease-causing organisms (Henriksson and Stacy, 1971). The molecular mechanisms responsible for the effects of rumen recovery or adaptation following SARA-mediated epithelial damage have not been determined.
addition, since butyrate has been shown to be beneficial in models of gastrointestinal disease in a variety of species (Segain et al., 2000; Huuskonen et al., 2004; Borthakur et al., 2008; Hanner et al., 2008; Thibault et al., 2010), we sought to also determine the efficacy of exogenous butyrate administration by studying changes in rumen epithelial gene expression. To this end, a microarray analysis, followed by qRT-PCR confirmation was completed based on information from existing databases to determine the importance of any affected metabolic pathways at the molecular level. In this study it was found that the observed changes in gene expression could be grouped into three categories: 1. NSHD genes (LCN2, MMP1, MUC16, GPX2, CSTA, FUT1, SERPINE2, PARDE6B, ADA, HPGD and TCHH), 2. RM Genes (BCAM, RAC3, MTOR, ACVR2A, LAMB1, FRS2, PPARD, DEDD2 and BMP6) and 3. Genes involved in the IR (NFKBIZ, NFKB2, LBP, NEDD4L, MAP3K8 and PLIN2). The individual genes, gene expression, role and contextual meaning are presented in Table 7.

NSHD genes are involved in conferring general protection to the organism and can take the form of increased barrier integrity, mucus production and reduced pathogen viability or attachment (Perez and Gipson, 2006). For example, by acting to sequester soluble Fe³⁺ from microbial siderophores, increased LCN2 expression can reduce the rate of microbial growth (Flo et al., 2004; Schmidt-Ott et al., 2007). Increased CSTA and GPX2 expression promote the maintenance and integrity of epithelial tight junctions (Blaydon et al., 2011) and limit the damaging effects of reactive oxygen species secreted by microorganisms (Brigelius-Flohe and Kipp, 2012). As such, they prevent the infiltration of potentially pathogenic bacteria into the deeper epithelial layers. Mucous layers are known lubricating barriers in epithelial cells and are a necessary component in the prevention of pathogen attachment (Perez and Gipson, 2008). FUT1 is an epithelial cell-surface receptor for pathogenic strains of E. coli and changes in its expression are thought to modulate pathogen attachment to epithelial cells (Yan et al., 2003; Wang et al., 2012) as is the expression of SERPINE2 (Luo et al., 2011). MUC16 which was highly elevated in this study is a glycosylated matrix protein known to provide a protective role in epithelial tissues (Perez and Gipson, 2008). PARDE6B is one of a group of genes responsible for attachment and polarity of epithelial cells to basolateral membranes (Suzuki et al., 2001). In this study, PARDE6B was downregulated which is in agreement with Gao et al. (2002) who found an inverse correlation between PARDE6B expression and tight junction integrity. ADA is a ubiquitous enzyme which is needed for the development and maintenance of the immune system (Wilson et al., 1991). In studies where ADA expression was repressed or suppressed entirely, the immune response was limited and under full suppression, forms the biological basis for Severe Combined Immunodeficiency (SCID) mice (Martin Jr. and Gelfand, 1981; Wilson et al., 1991). ADA was reduced in our study helping to reduce the extent and severity of the immune response. HPGD, an enzyme responsible for prostaglandin metabolism, was reduced in our study. Prostaglandins can have diverse roles depending on the presence of disease. For example, Cyclooxygenase-1 (COX1) is constitutively expressed in a normal gastrointestinal tract and is responsible for the expression of protective prostaglandins, namely PGE2. COX2 is inducible and its expression rapidly increases in response to pathogenic stimuli (Cho et al., 2006; Wallace, 2008). It follows then that the lower expression of HPGD seen in this study can lead to protective effects in the rumen by controlling inflammation and helping to maintain levels of COX1 metabolites.

Genes involved in remodelling or adaptation are those that are responsible for the degradation of the extracellular matrix, its reorganization, cellular movement, signalling and adaptation to new metabolic requirements. The particulars of ECM reorganization with respect to wound healing have been reviewed extensively (Schreml et al., 2010). Briefly, ECM reorganization follows a tightly choreographed series of events initiated by the degradation of existing ECM proteins, followed by clearance, the deposition of a new matrix milieu and proliferation and differentiation of epithelial cells favouring the new conditions. MMP1 (+21.6) is a matrix metallopeptidase secreted by subepithelial fibroblasts in response to the need for a rearrangement of extracellular architecture in response to injury, growth, or adaptation (Birkedal-Hansen et al., 1993; Pendas et al., 1996; 1997). These events involve a wide variety of genes. In this study, BCAM (a receptor for matrix proteins) was elevated in addition to RAC3, which together aid in the remodeling process (Haataja et al., 2002; Eyler and Telen, 2006). It is unknown how long the early phases of remodeling occur in rumen epithelium. However, evidence from this study suggests that on the day the biopsies were taken for analysis, the animals were still in the early phase of remodeling. This can be seen in the downregulation of LAMB1 (Taniguchi et al., 2009), BMP6 (Kaiser et al., 1998), ACVR2A (Lebrun et al., 1999; Tsuchida et al., 2004), PPARD (Tan et al., 2007), TCHH (Steinert et al., 2003) and in the downregulation of MTOR, which has been shown to be necessary for ECM degradation (Sarbassov et al., 2004; Ong et al., 2007).
Fig. 1. Context-specific pathway analysis of genes upregulated (green) or downregulated (red) due to the addition of exogenous butyrate supplementation at 2.5% DMI. Panels A, B, and C represent genes involved in Non-Specific Host Defense (NSHD), remodeling, or the Immune Response (IR) respectively. Genes coloured gray indicate no change while those in white are present as proposed pathway intermediates where necessary, but were not represented in the analysis.

In a similar fashion, FRS2, which plays an integral role in cellular adhesion and growth (Ong et al., 2000; Norambuena and Schwartz, 2011), was downregulated and thus likely contributed to the degradation and reorganization of the ECM. Finally, during the adaptive process, recovering healthy epithelium is induced to proliferate. An anti-proliferative factor, DEDD2 was downregulated in this study. Apoptosis is usually seen in polymorphonuclear cells following the inflammatory phase of wound healing in skin models (Lee et al., 2002; Eming et al., 2007).

IR genes are general indicators of immune system status and stimulation. Indeed, a continued and prolonged immune response such as that seen in SARA, can be detrimental to the growth and maintenance of an organism. In fact, the partitioning of nutrients for the execution of an immune response is well known (Dionissopoulos et al., 2001; Meazza et al., 2004; Li et al., 2006). One of the most important ways in which tissues regulate the immune response is through the activation or suppression of NF-κB signalling pathways (Gilmore, 2006). In this study, all genes in the IR category were downregulated by the addition of exogenous butyrate. Most of these are upstream effectors of NF-κB (AKIRIN2, NFKBIZ, NFKB2) (Cowland et al., 2006; Totzke et al., 2006; Goto et al., 2008; Al-Sadi et al., 2010). Others such as LBP (-2.0-fold), require binding of free LPS to its receptor, TLR4 for downstream signalling events to take place (Gray et al., 1993). In addition, Li et al. (2011) were able to determine that NEDD4L is a positive regulator of NF-κB which is in agreement with our results. Similarly, the ability of MAP3K8 and PLIN2 to activate the NF-κB pathway has been documented (Chan and Reed, 2005; Cismasiu et al., 2009; Mattos et al., 2010). These direct relationships are supported by results presented here.

All expression data was compiled and analyzed for pathway interactions using the Ingenuity Systems™ KEGG database (Tanabe and Kanehisa, 2012; Zhou, 2013). As in Table 7, the PCR data was placed in the three functional categories of NSHD, RM and IR and hypothetical pathway diagrams were constructed for each based on experimental findings of the response to LPS and exogenous butyrate following a grain challenge. These figures clearly demonstrate the central role of butyrate in our experimental model. Figure 1A indicates the beneficial effects of Butyrate on NSHD. Figure 1B represents the effect of butyrate on the modulation of the rumen Epithelial Extracellular Matrix (ECM) and Fig. 1C shows the downregulation of the immune response to LPS following butyrate administration. Butyrate has been well known as a Histone Deacetylase (HDAC) inhibitor and its effects in attenuating inflammatory
conditions have been hypothesized to be the result of modulation of the NF-κB signalling cascade at multiple levels (Miyoshi et al., 2011). TLR4/NF-κB controls the expression of many inflammatory genes (Fig. 1C) and so it makes sense that it can serve as a control point for limiting the IR (Suuronen et al., 2003). Indeed, several studies have reported a decrease in the TLR4/NF-κB response to LPS following butyrate administration (Huuskonen et al., 2004; Wu et al., 2012). In addition to reductions in NF-κB family genes, we propose that butyrate limits the extent of the immune response to LPS by reducing the expression of additional genes shown to be effectors of the IR. Although we have placed the effects of butyrate in three different categories, it was our intent to demonstrate that butyrate mediates its beneficial effects through the differential expression of multiple genes related to NSHD, remodelling and the IR within epithelial cells exposed to LPS.

To our knowledge, this type of analysis has not been conducted on rumen epithelium adapting to an acidic diet in response to exogenous butyrate. As with any new therapeutic intervention, it is not known if its administration will have negative effects on the well-being on the host. This type of approach allows the quantification of such findings and demonstrates the viability of butyrate supplementation in the treatment of SARA in response to a high grain/high energy diet.

5. CONCLUSION

Although the data presented here indicated that butyrate supplementation had little effect on milk parameters and pH, it can be seen that the extent of the immune response in SARA is further limited by reductions in the expression of the acute phase protein, LPS. In addition, gene expression data clearly shows the enhancement of preparative and compensatory mechanisms in SARA facilitated by butyrate. The advanced pathways explored here represent potential biomarkers and indicate the pervasive and yet benign nature of butyrate supplementation. It can be seen that exogenous butyrate reduces the expression of key inflammatory markers, enhances non-specific cellular defences to microorganisms and the remodelling of the ECM to favour adaptation to a high grain/high energy environment.

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