Individual animal variability in ruminal bacterial communities and ruminal acidosis in primiparous Holstein cows during the periparturient period

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ABSTRACT

The purpose of this study was to investigate variability among individual cows in their severity of ruminal acidosis (RA) pre- and postpartum, and determine whether this variability was related to differences in their ruminal bacterial community composition (BCC). Variability in the severity of RA among individual cows was characterized based on ruminal fermentation variables. Effects of prepartum dietary treatment on the severity of RA were also examined. Fourteen Holstein heifers paired by expected calving date and BCS were allotted to 1 of 2 prepartum dietary treatments: low-concentrate or high-concentrate diets. All cows received the same lactation diet postpartum. Microbial DNA extracted from 58 ruminal digesta samples in total collected prepartum (d -50, -31, and -14; 27 samples) and postpartum (d +14 and +52; 31 samples) and amplified by PCR were subjected to automated ribosomal intergenic spacer analysis. Changes in ruminal variables over time [pH, volatile fatty acids (VFA), and acidosis indicators, including duration and area under the rumen pH curve below 5.8, 5.5, and 5.2, measured on d -54, -35, -14, -3, +3, +17, +37, and +58] were analyzed using principal components analysis. Based on the shift (defined as the distance of the mean loadings) between the prepartum and postpartum period for each cow, the 14 cows were classified into 3 groups: least acidotic ($n = 5$), most acidotic ($n = 5$), and intermediate ($n = 4$). Cows in the most acidotic group had greater severity of RA (measured as duration of total RA, mild RA, moderate RA, and acute RA; area under the pH curve for total RA, mild RA, and moderate RA) postpartum than prepartum, and this difference between periods was greater than for the least acidotic cows. Similarly, the RA index (total area of pH <5.8 normalized to intake) showed an interaction between severity of RA and period. The variation in the severity of RA was

independent of intake, total VFA concentration, and individual VFA proportions. Production variables (milk yield, fat percentage, fat yield, fat-corrected milk, and efficiency of milk production) were not influenced by the severity of RA. Ruminal BCC was not influenced by dietary treatment or period. However, some cows experienced greater shift in BCC than other cows across the periods. Based on the magnitude of the shift in BCC (distance between mean ordination values across the periods for each cow), cows were grouped into 3 BCC profile categories: stable (5 cows with lesser shift), unstable (5 cows with greater shift), and intermediate (4 cows with average shift). Cows demonstrating a greater shift in BCC were not necessarily those in the most acidotic group and vice versa. The shift in ruminal fermentation variables (principal components analysis rankings) and the shift in BCC (automated ribosomal intergenic spacer analysis rankings) between pre- and postpartum were not related ($n = 14$; $R^2 = 0.00$). It was concluded that not all cows are equally susceptible to RA and postpartum shifts in BCC appear to be independent of the differences in the severity of RA postpartum.

Key words: individual animal variability, periparturient period, ruminal acidosis, ruminal bacterial community

INTRODUCTION

During the transition period, the energy requirement of the cow increases to support the growth of the fetus and the energy demand for lactation following calving (Drackley, 1999). However, the depression in feed intake that occurs in the prefresh period coupled with higher energy demand induces a negative energy balance, which increases the susceptibility of cows to metabolic disorders (Hayirli et al., 2002; Ingvarsen, 2006). Several nutritional strategies have been proposed to minimize the incidence of metabolic disorders such as fatty liver and ketosis (Ingvarsen, 2006; Janovick et al., 2011) and to improve DMI during the transition period (Ingvarsen and Andersen, 2000; Penner and Oba, 2009;

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Janovick and Drackley, 2010). One of the strategies that has received considerable attention is to provide a more fermentable diet during the prepartum period to facilitate the growth of ruminal papillae (Dirksen et al., 1985) and, thus, help the cows adapt to the high-energy diet fed postpartum (NRC, 2001). However, the large reduction in DMI before calving (Hayirli et al., 2002) and the subsequent increase in DMI in the first few weeks postpartum increases the susceptibility of cows to SARA (Nocek, 1997; Penner et al., 2007).

Considerable variation has been reported to exist among individual cattle in their severity of ruminal acidosis (**RA**). In a study that examined the fractional rates of VFA absorption in cows fed a low- (8%) or high- (64%) concentrate diet, Penner et al. (2009b) observed greater variation in ruminal pH among cows fed the high-concentrate diet. They reported that 1 out of the 6 cows on the high-concentrate diet had markedly higher mean pH (6.19) and a lower acidosis index (area of pH <5.8 $\times$ min/kg of DMI) than the other cows. Another study (Bevans et al., 2005), comparing the effect of a rapid dietary transition (increasing from 40 to 90% concentrate in 3 d with 1 intermediate diet) to that of a gradual transition (40 to 90% concentrate in 15 d with 5 intermediate diets fed 3 d each) protocol in growing beef cattle, observed a range of individual responses to grain challenge with both protocols. Some animals on the rapid-adaptation protocol managed uneventful transition to finishing diets, whereas some animals experienced acidosis even when they were on the gradual-adaptation protocol. Although Penner et al. (2009a) demonstrated that sheep with greater rates of acetate and butyrate uptake are at lower risk than those with lower rates, few studies have sought to determine the causes for the variation in severity of RA among individual cattle.

It is well known that ruminal bacterial communities respond to changes in diet and environmental conditions (Dehority and Orpin, 1997; Dehority, 2003) and also exhibit host specificity (Weimer et al., 2010a). Despite the importance for understanding microbial changes in transition dairy cattle, no published studies exist, to our knowledge, that have evaluated shifts in ruminal bacterial community composition (**BCC**) in dairy cows during the transition period.

The present study is an extension of a study (Penner et al., 2007) in which the effect of additional concentrate allocation prepartum on postpartum RA was evaluated using 2 feeding regimens: (1) control (**Ctrl**) treatment reflecting the NRC (2001) recommendations for transitioning a pregnant heifer into lactation and (2) high-concentrate (**HC**) treatment. In that study, the severity of RA was greater postpartum than prepartum, but was not influenced by the dietary regimens. However,

large variability existed among cows within each treatment for the severity of RA. Thus, the objectives of the present study were to (1) explore the differences in severity of RA pre- and postpartum among individual cows and relate this variation to differences in ruminal fermentation, intake, milk production, and proportions of ruminal bacteria; (2) determine the effect of treatment and period on ruminal BCC and the shifts in BCC among individual cows across the periods; and (3) investigate if the cows experiencing greater shifts in BCC had greater severity of RA and vice versa.

MATERIALS AND METHODS

Experimental Design and Data Collection

Experimental details were previously reported (Penner et al., 2007). In brief, 14 ruminally cannulated Holstein heifers paired by expected calving date and BCS were used in a completely randomized design and assigned to 1 of 2 prepartum feeding regimens: Ctrl and HC treatments. The Ctrl treatment consisted of a far-off diet [forage:concentrate (**F:C**) = 80:20] fed from d -60 to -25 and a close-up diet (F:C = 54:46) fed from d -24 to parturition. The HC feeding regimen consisted of 4 prepartum diets: F:C = 68:32 fed from d -60 to -43, F:C = 60:40 fed from d -42 to -25, F:C = 52:48 fed from d -24 to -13, and F:C = 46:54 fed from d -12 to parturition. All cows received the same lactation diet postpartum (F:C = 46:54). A detailed ingredient and chemical composition of the diets is tabulated (Table 1).

Ruminal pH was continuously measured (every 30 s) for 3 consecutive days starting on d -54 $\pm$ 3.9, d -35 $\pm$ 4.1, d -14 $\pm$ 4.4, d -3 $\pm$ 0, +3 $\pm$ 0, d +17 $\pm$ 1.2, d +37 $\pm$ 1.4, and d +58 $\pm$ 1.5 (means $\pm$ SD) relative to calving using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor Inc., Escondido, CA) as described by Penner et al. (2006). Minimum ruminal pH, maximum pH and mean pH were calculated from the daily pH data averaged by minute. The severity of RA was characterized based on 3 pH thresholds: 5.8, 5.5, and 5.2 (Maekawa et al., 2002; Beauchemin and Yang, 2005). The severity of RA was described as mild (when 5.8 > ruminal pH > 5.5), moderate (when 5.5 > ruminal pH > 5.2), and acute (when ruminal pH < 5.2). Total RA represents the sum total of RA over a day that pH remained below 5.8. For each category of RA, the duration (h/d) and total area (pH $\times$ minute) that pH remained below each threshold was calculated. Additionally, the number of daily episodes of each category of RA was also recorded. Ruminal fluid collected at 1630 h on 2 consecutive days starting on d -54 $\pm$ 3.3, d -36 $\pm$ 5.2, d -13 $\pm$ 4.5, d -3 $\pm$ 0, d +3 $\pm$ 0, d

Table 1. Ingredient and chemical composition of the experimental diets (DM basis)

Item	Treatment ¹						Lactation diet
	Ctrl		HC				
	Far-off	Close-up	HC-1	HC-2	HC-3	HC-4	
Ingredient composition ² (% of DM)							
Forage							
Barley silage	69.1	47.0	58.8	52.2	45.9	40.6	42.2
Alfalfa hay	—	7.2	—	—	—	—	4.4
Grass hay	11.7	—	8.7	7.7	6.0	6.0	—
Concentrate							
Steam-rolled barley	6.7	9.1	—	—	—	—	28.6
Ground barley	2.2	4.5	17.1	18.0	21.4	22.3	2.2
Canola meal	5.3	4.5	5.5	4.2	5.8	5.5	1.8
Corn gluten meal	1.6	3.6	—	—	—	—	2.9
Dry corn distillers grains	—	—	0.4	0.8	6.4	4.9	—
Wheat middlings	—	—	4.2	8.0	5.1	7.8	—
Beet pulp	—	13.6	4.2	8.0	5.1	7.8	2.2
Calcium carbonate	0.9	1.8	0.8	0.8	0.8	0.8	0.6
Calcium diphosphate	0.6	0.7	—	—	—	—	0.7
Sodium bicarbonate	—	—	—	—	—	—	0.6
Magnesium oxide	—	0.4	—	—	—	—	0.1
Megalac ³	—	—	—	—	—	—	2.0
Beet molasses	1.3	0.9	—	—	—	—	1.7
Canola oil	0.2	0.5	0.1	0.1	0.1	0.1	0.5
Nutrichlor ⁴	—	5.9	—	—	3.2	4.0	—
Vitamin-mineral mix	0.4	0.3	0.2	0.2	0.2	0.2	0.7
SoyPass ⁵	—	—	—	—	—	—	8.8
Chemical composition							
DM (%)	50.4	56.5	59.3	59.2	63.2	63.7	57.5
OM (% of DM)	91.3	91.2	92.3	92.6	92.6	92.3	91.5
CP (% of DM)	14.0	16.7	14.9	14.9	17.8	16.4	17.3
NDF (% of DM)	36.4	31.3	33.2	33.7	30.6	30.4	29.4
ADF (% of DM)	19.2	18.3	16.9	16.9	15.6	15.5	15.6
NFC ⁶ (% of DM)	39.6	42.5	43.3	43.1	43.4	44.9	43.5
Predicted NE _L ⁷ (Mcal/kg)	1.41	1.50	1.53	1.55	1.58	1.59	1.72

¹The control (Ctrl) treatment consisted of a far-off diet that was fed from d –60 to –25 and a close-up diet fed from d –24 d to parturition. The high-concentrate (HC) treatment consisted of 4 diets: HC-1 was fed from d –60 to –43, HC-2 was fed from d –42 to –25, HC-3 was fed from d –24 to –13, and HC-4 was fed from d –12 to parturition. Cows on both treatments received the same diet postpartum.

²Formulated to contain 0.81% Ca, 0.41% P, 0.23% Mg, 1.2% K, 0.25% S, 0.30% Na, 1.0% Cl, 145 mg of Fe/kg, 60 mg of Zn/kg, 18 mg of Cu/kg, 53 mg of Mn/kg, 0.3 mg of Se/kg, 0.6 mg of Co/kg, 0.3 mg of I/kg, 8.3 kIU of vitamin A/kg, 1.3 kIU of vitamin D/kg, and 62.7 IU of vitamin E/kg (DM basis).

³Megalac calcium salts of palm oil (Church and Dwight Co. Inc., Princeton, NJ).

⁴Nutritech Solutions Ltd. (Abbotsford, BC, Canada).

⁵LignoTech USA Inc. (Rothschild, WI).

⁶Average values for ether extract and neutral detergent insoluble nitrogen were used in the calculation (NRC, 2001).

⁷Calculated using equations from NRC (2001).

+17 ± 1.2, d +37 ± 1.4, and d +58 ± 1.5 (means ± SD) relative to calving were used for determining VFA concentrations as described by Penner et al. (2007).

Ruminal fluid samples for determining BCC were collected 5 times from each cow during the transition period: three times prepartum (d –50 ± 4.0, –31 ± 5.9, –14 ± 2.6; means ± SD) and twice (d +14 ± 5.4 and +52 ± 8) postpartum. Approximately 750 mL of ruminal fluid was collected from 3 different locations in the rumen (reticulum, ventral rumen sac, and the interface between the fluid phase and the ruminal mat) at 1630 h (3 h postfeeding) and strained through perforated material (Peetex, pore size = 355 µm; Sefar

Canada Inc., Scarborough, ON, Canada). An aliquot of 2 mL was then transferred to a 3-mL polypropylene tube (BD Biosciences, San Jose, CA) and centrifuged at 8,000 × *g* for 20 min. The supernatant was discarded and 2 mL of storage buffer (ASL buffer; Qiagen Inc., Mississauga, ON, Canada) was added to the cell pellet and stored at –80°C for DNA extraction later.

Microbial DNA was extracted from the liquid phase of ruminal digesta as described by Stevenson and Weimer (2007). In brief, DNA was extracted using a series of wash steps with the extraction buffer, followed by lysis of the microbial cells in a bead beater, extraction with combinations of phenol/chloroform, and precipitation

with isopropanol (Stevenson and Weimer, 2007). The DNA obtained was resuspended in Tris-EDTA (10 mM Tris HCl and 1 mM EDTA, pH 8.0) and its concentration measured spectrophotometrically.

The internally transcribed region (**ITS**) between the bacterial 16S and 23S rRNA genes was amplified using domain-specific bacterial primers ITSF (5'-GTCGTAACAAGGTAGCCGTA-3') and ITSReub (5'-GCCAAGGCATCCAAC-3'). The primer ends were complementary to the respective positions 1423 and 1443 of the 23S rRNA and positions 38 and 23 of the 16S rRNA of *Escherichia coli* (Cardinale et al., 2004). The PCR components and the cycling conditions have been described previously (Weimer et al., 2010b). The PCR product obtained was resolved in a Beckman Coulter CEQ8000 Genetic Analysis System using the run parameters as described by Weimer et al. (2010b). In brief, 0.5 µL of the PCR product was mixed with 1 µL of Beckman Coulter WelRed #1 infrared fluorescent dye-labeled DNA standard ladder (MapMarker 1000; BioVentures Inc., Murfreesboro, TN) and 39 µL of sample loading solution (Beckman Coulter), and loaded into microtiter plates. Capillary electrophoresis was conducted following the manufacturer's instructions after covering the liquid surface of the wells with molecular biology-grade mineral oil.

The raw data obtained from capillary electrophoresis were imported into GeneMarker software (version 1.75; Soft Genetics LLC, State College, PA) for further analysis. Peak detection and quantification was performed using the settings for amplicon fragment length polymorphism analysis described in the GeneMarker manual. Peak sizes (bp) were determined using the DNA standard ladder described above. Baseline subtraction and peak smoothing were performed as described in the GeneMarker manual. The panel generated by the software was screened manually to remove any questionable peaks caused by pull-up from the dyed DNA standard. All peaks corresponding to amplicon length >112 bp were exported for correspondence analysis (described in the Statistical Analysis section).

Quantitative real-time PCR assays were conducted using the DNA extracted from the liquid phase of ruminal digesta, using POWER SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), forward and reverse primers (25 pmol of each primer/reaction) and approximately 20 ng of template DNA in a final volume of 25 µL per reaction. Quantitative real-time PCR assays were conducted using Applied Biosystems Prism 7300 sequence detection system. The primers for *Megasphaera elsdenii* (MegEls2F 5'-AGA TGG GGA CAA CAG CTG GA-3' and MegEls2R 5'-CGA AAG CTC CGA AGA GCC T-3') and *Prevotella* (PreGen4F 5'-GGT TCT GAG AGG AAG GTC CCC-3' and Pre-

Gen4R 5'-TCC TGC ACG CTA CTT GGC TG-3') used for the assay have been validated for specificity (Stevenson and Weimer, 2007). The amplification conditions were 40 cycles of 95°C for 15 s and an annealing and extension period of 60 s at 59°C. The PCR product specificity was verified by melt denaturation and PCR efficiency was calculated as the negative reciprocal of the slope of the line obtained by plotting Ct versus log DNA concentrations of the standard dilution series. Standards for *M. elsdenii* and genus *Prevotella* were prepared by making a serial dilution of the genomic DNA from pure cultures of *M. elsdenii* strain T81 and *Prevotella brevis* strain GA33, respectively. Standards for domain bacteria were prepared from bacterial DNA recovered from ruminal samples. The standards and the set of samples belonging to each period were run in the same plate in triplicate. The relative population size (**RPS**) of the target bacterium was determined as the ratio of the amplification of target taxon (e.g., *M. elsdenii*) 16S rRNA copy numbers to the amplification of the background obtained by amplifying the 16S rRNA gene with eubacterial primers (BAC338F and BAC805R; Yu et al., 2005); details of these calculations, with corrections for PCR efficiency, have been described by Stevenson and Weimer (2007).

Statistical Analysis

The data matrix resulting from the export of peak areas from GeneMarker were analyzed by correspondence analysis following the method of Ludwig and Reynolds (1988) using custom software written in the C programming language. The ordination points for the first 2 components were plotted as scatter plots. The relative peak areas obtained from automated ribosomal intergenic spacer analysis (**ARISA**) were analyzed for the shifts in BCC between treatments as well as between periods using analysis of similarity (**ANOSIM**; Clarke, 1993). Mean ordinations were calculated by period from the eigenvalues of the first 2 correspondence components for each cow to determine the shift in BCC (distance) from prepartum to postpartum period (McCune and Grace, 2002). Based on the shift in the ordination points from prepartum to postpartum for each cow, the 14 cows were ranked and classified as cows with stable ARISA profile (lesser shift between periods), intermediate profile (intermediate shift between periods), and unstable profile (greater shift between periods).

The relationships among the ruminal pH variables (minimum pH, mean pH, and maximum pH), variables measuring the severity of RA (duration and area under the pH curves representing mild RA, moderate RA, acute RA, and total RA; number of daily episodes the

ruminal pH remained below the thresholds 5.8, 5.5, and 5.2), intakes and ruminal fermentation variables (total VFA concentration and proportions of acetate, propionate, and butyrate) were evaluated from the loading plots of principal components (PC) analysis (PCA), based on the correlation matrix (consisting of 22 variables), using PROC PRINCOMP and PROC CORR of SAS (version 9.1; SAS Institute Inc., Cary, NC).

Mean loadings were calculated by period from the eigenvectors of PC1 and PC2 for each cow to determine the shift in ruminal metabolism (distance) due to changes in ruminal fermentation and acidosis from prepartum to postpartum period. Based on the distance of the mean loadings between the prepartum and postpartum period for each cow, the 14 cows were ranked and classified into 3 groups: least acidotic (LA; lesser shift between prepartum and postpartum period), most acidotic (MA; greater shift between prepartum and postpartum period), and intermediate (IN; intermediate shift between prepartum and postpartum period). With the grouping of the cows as described, the main effects of severity of RA, period (pre- and postpartum), and their interactions were determined using PROC MIXED of SAS. Intake, ruminal fermentation and acidosis variables, and RPS of *Prevotella* and *M. elsdenii* averaged by period for each cow (to obtain prepartum and postpartum means for each variable) were included in the model. To determine if severity of RA was related to milk production variables (yield, fat percentage, fat yield, FCM, and production efficiency) or RPS of *Prevotella* and *M. elsdenii* (data from postpartum period), these variables were also analyzed using PROC MIXED of SAS. Least squares means for severity of RA were reported and declared significant when $P < 0.05$.

RESULTS

BCC

The total number of amplicons (ranging in size from 62 to 815 bp) detected by the analysis of raw data generated from capillary electrophoresis of PCR products was 222. The mean number of amplicons detected in individual samples was 106, ranging from a minimum of 59 to a maximum of 129. The mean number of amplicons detected in the ruminal fluid during the prepartum and postpartum period was 105 and 111, respectively, for HC cows and 105 and 105, respectively, for Ctrl cows. Correspondence analysis of the ARISA profile across the entire data set revealed that the first 2 components contributed 6 and 3.6%, respectively, to the total variation in the profile.

Figure 1. Scatter plot of the ordination points obtained from correspondence analysis of the automated ribosomal intergenic spacer analysis (ARISA) data matrix from the liquid phase of rumen digesta. The open and filled circles represent the ordination points corresponding to the control or low-concentrate treatment (Ctrl; $n = 31$) and high-concentrate treatment (HC; $n = 27$), respectively. Data points represent samples from 14 cows across 5 periods (3 periods prepartum and 2 periods postpartum).

The ordination points representing the dietary treatments (Figure 1) and the period within a treatment (Figure 2a and 2b) did not cluster separately. Analysis of similarity for ARISA profiles showed no differences between treatments (ANOSIM R value = -0.11 ; +1 indicates maximum differences and -1 indicates maximum similarity) or among periods within a treatment (ANOSIM R value for Ctrl = -0.347 ; HC = -0.408). However, variability existed in the magnitude of shift in BCC among cows across the periods (Table 2).

Correlations and PCA

Correlation coefficients for PCA are presented in Table 3. Ruminal pH variables (minimum pH, mean pH, and maximum pH) were positively correlated with one another and with ruminal acetate proportion and the acetate-to-propionate ratio. Ruminal pH variables were negatively correlated with ruminal propionate proportion, whereas the correlations between acetate and propionate proportions were strongly negative ($r = -0.92$; $P < 0.01$). Without any exceptions, all of the variables measuring the severity of RA [mild RA duration and area, moderate RA duration and area, acute RA duration and area, total RA duration and area, and daily episodes less than the threshold pH (5.8, 5.5, and 5.2)] were negatively correlated with the ruminal pH variables. With a few exceptions (daily episodes pH < 5.8 and mild RA duration), all of the variables measuring the severity of RA were positively correlated with one another. Dry matter intake was weakly but positively correlated with total VFA concentration

Figure 2. Scatter plot of the ordination points obtained from correspondence analysis of the automated ribosomal intergenic spacer analysis (ARISA) data matrix from the liquid phase of rumen digesta. In this figure, the ordination points corresponding to the prepartum and postpartum periods were plotted separately for each treatment (a: control or low-concentrate treatment, Ctrl, $n = 31$; b: high-concentrate treatment, HC, $n = 27$). Both figures have the same scale. Note that the open and closed symbols representing prepartum and postpartum periods for each diet are intermingled, indicating a lack of period effect on ruminal bacterial community composition.

($r = 0.39$; $P < 0.01$) and propionate proportion ($r = 0.40$; $P < 0.01$), weakly but negatively correlated with minimum pH ($r = -0.22$; $P < 0.05$) and maximum ruminal pH ($r = -0.23$; $P < 0.05$), and not correlated with any of the variables measuring the severity of RA, with the exception of a weak positive correlation with daily episodes pH < 5.8 ($r = 0.28$; $P < 0.01$). Total VFA concentration showed weak negative correlations with pH variables and positive weak correlations with mild RA duration, daily episodes pH < 5.8 , and ruminal propionate proportion. The correlations of ruminal propionate proportion with the variables measuring mild to moderate RA were relatively strong and positive compared with its correlations with the variables measuring acute acidosis (acute RA duration and acute RA area). It should be noted that ruminal butyrate proportion was not correlated with any of the variables,

with the exception of a weak negative correlation with propionate proportion ($r = -0.25$; $P < 0.05$).

Principal components analysis of the variables influencing ruminal fermentation and acidosis (individual and total VFA, ruminal pH variables, and the variables measuring the severity of RA) revealed that the first 3 components contributed 52, 19, and 6%, respectively, whereas the sum of the remaining components (Σ PC4 to PC22) contributed a total of 23% to the variability in ruminal fermentation and RA (Table 4). The first PC had high loadings for the ruminal pH variables, mild RA duration, moderate RA duration, acute RA duration, total RA duration, moderate RA area, acute RA area, and daily episodes for pH < 5.5 and 5.2 (Table 5). The second PC had high loadings for ruminal acetate proportion, propionate proportion, acetate-to-propionate ratio, mild RA area, total RA area, and daily episodes for pH < 5.8 . The third PC had high loadings for DMI, total VFA concentration, and butyrate proportion. Thus, all of the data were adequately described by the first 3 PC. Three clusters could be identified from the loading plot of PC1 and PC2 (Figure 3a). The cluster in quadrant A consisted of ruminal pH variables and the cluster in quadrant B included mild RA area, moderate RA area, acute RA area, total RA area, duration pH < 5.5 , area under the pH curve below 5.5, and daily episodes of ruminal pH < 5.2 . The cluster in quadrant C consisted of mild RA duration, moderate RA duration, total RA duration, daily episodes of ruminal pH < 5.8 and 5.5 , and ruminal propionate. Principal component 1 separated ruminal pH variables (positively correlated with each other) from the variables describing the severity of RA and propionate proportion, indicating the negative correlations between ruminal pH variables and the variables measuring the severity of RA. Principal component 2 separated the proportion of acetate from that of propionate and also the variables describing the severity of RA. Dry matter intake, total VFA concentration, and butyrate proportion were described by PC3 with high loadings (Figure 3b).

A score plot of the first 2 PC by treatment showed no differences between Ctrl and HC treatment (Figure 4). When each treatment was plotted by period, the majority of the data points clustered by period for each diet, indicating differences in ruminal fermentation and acidosis before and after calving (Figure 5a and 5b). However, variability existed among cows with the shift in the mean loadings for each cow across the periods ranging from 0.7 to 7.8 (Table 2). The relationship between the shifts in ruminal fermentation variables (PCA rankings) and the shifts in BCC (ARISA rankings) was not significant ($n = 14$; $r^2 = 0.00$; $P = 0.98$; Figure 6).

Table 2. Shifts in ruminal parameters¹ and ruminal bacterial communities between the prepartum and postpartum period

Severity of RA ²	Cow identification	Treatment ³	Shift in PCA profiles ⁴	PCA rank	Stability ⁵	Cow identification	Treatment	Shift in ARISA profiles ⁶	ARISA rank
LA	111	HC	0.7	1	Stable	817	HC	0.2	1
	863	Ctrl	2.6	2		110	Ctrl	0.5	2
	104	Ctrl	3.0	3		820	Ctrl	0.7	3
	1008	Ctrl	3.4	4		261	HC	1.0	4
	261	HC	3.4	4		1008	Ctrl	1.2	5
IN	110	Ctrl	3.7	6	Intermediate	863	Ctrl	1.4	6
	844	HC	4.2	7		9671	HC	1.5	7
	879	HC	4.8	8		194	HC	1.7	8
	9671	HC	4.8	8		844	HC	1.7	8
	213	Ctrl	5.2	10		104	Ctrl	1.8	10
MA	194	HC	5.2	10	Unstable	111	HC	1.9	11
	817	HC	5.6	12		879	HC	2.0	12
	217	Ctrl	7.8	13		217	Ctrl	2.3	13
	820	Ctrl	7.8	13		213	Ctrl	3.2	14

¹Ruminal parameters include pH variables (minimum pH, mean pH, and maximum pH), variables measuring severity of ruminal acidosis (RA; mild RA duration and area, moderate RA duration and area, acute RA duration and area, total RA duration and area, and daily episodes where pH remained <5.8, <5.5, and <5.2), DMI and rumen fermentation parameters (total VFA, acetate, propionate, and butyrate).

²LA = least acidotic group; IN = intermediate group; MA = most acidotic group.

³The control (Ctrl) treatment consisted of a far-off diet that was fed from d −60 to −25 and a close-up diet fed from d −24 to parturition. The high-concentrate (HC) treatment consisted of 4 diets: HC-1 was fed from d −60 to −43, HC-2 was fed from d −42 to −25, HC-3 was fed from d −24 to −13, and HC-4 was fed from d −12 to parturition. Cows on both treatments received the same diet postpartum.

⁴Distance between the mean principal components analysis (PCA) loadings for each cow across the periods.

⁵Automated ribosomal intergenic spacer analysis (ARISA) profiles with lesser shift (stable), intermediate shift (intermediate), and greater shift (unstable) across the periods.

⁶Distance between the mean ordination values for each cow across the periods as determined by correspondence analysis.

Table 3. Correlations among the variables influencing ruminal fermentation and ruminal acidosis (RA) parameters

Variable ¹	Min pH	Mean pH	Max pH	Mild RA duration	Mild RA area	Mod RA duration	Mod RA area	Acute RA duration	Acute RA area	Total RA duration	Total RA area	Duration pH <5.5	Area pH <5.5
Min pH	1.000												
Mean pH	0.87**	1											
Max pH	0.50**	0.73**	1										
Mild RA duration (h/d)	-0.71**	-0.78**	-0.53**	1									
Mild RA area (pH × min)	-0.32**	-0.43**	-0.32**	0.19	1								
Mod RA duration (h/d)	-0.75**	-0.85**	-0.54**	0.71**	0.46**	1							
Mod RA area (pH × min)	-0.70**	-0.79**	-0.50**	0.45**	0.64**	0.87**	1						
Acute RA duration (h/d)	-0.62**	-0.69**	-0.41**	0.28**	0.68**	0.70**	0.96**	1					
Acute RA area (pH × min)	-0.55**	-0.60**	-0.31**	0.20*	0.64**	0.51**	0.78**	0.88**	1				
Total RA duration (h/d)	-0.82**	-0.92**	-0.59**	0.87**	0.46**	0.95**	0.83**	0.68**	0.53**	1			
Total RA area (pH × min)	-0.33**	-0.43**	-0.32**	0.19	0.99**	0.47**	0.64**	0.69**	0.64**	0.46**	1		
Duration pH <5.5 (h/d)	-0.76**	-0.85**	-0.53**	0.59**	0.59**	0.96**	0.97**	0.88**	0.70**	0.91**	0.59**	1	
Area pH <5.5 (pH × min)	-0.69**	-0.77**	-0.47**	0.40**	0.67**	0.81**	0.99**	0.98**	0.88**	0.78**	0.67**	0.94**	1
Episodes pH <5.8	-0.65**	-0.63**	-0.43**	0.77**	0.04	0.44**	0.25*	0.13	0.07	0.60**	0.05	0.35**	0.21*
Episodes pH <5.5	-0.74**	-0.78**	-0.44**	0.81**	0.29**	0.88**	0.67**	0.49**	0.34**	0.90**	0.29**	0.79**	0.61**
Episodes pH <5.2	-0.64**	-0.71**	-0.45**	0.37**	0.51**	0.77**	0.93**	0.89**	0.77**	0.73**	0.51**	0.88**	0.92**
DMI (kg/d)	-0.22*	-0.19	-0.23*	0.17	-0.17	0.11	0.09	0.06	0.00	0.15	-0.17	0.10	0.07
Total VFA (mM)	-0.40**	-0.33**	-0.28**	0.34**	-0.07	0.07	0.04	0.04	0.10	0.21*	-0.07	0.06	0.06
Acetate (mmol/100 mol)	0.55**	0.55**	0.38**	-0.62**	0.05	-0.54**	-0.33**	-0.19	-0.06	-0.58**	0.047	-0.44**	-0.27**
Propionate (mmol/100 mol)	-0.55**	-0.58**	-0.39**	0.65**	-0.03	0.58**	0.36**	0.21*	0.08	0.62**	-0.03	0.47**	0.30**
A:P ratio	0.59**	0.57**	0.36**	-0.65**	0.04	-0.54**	-0.33**	-0.19	-0.07	-0.59**	0.03	-0.44**	-0.27**
Butyrate (mmol/100 mol)	0.02	0.07	-0.01	-0.07	-0.02	-0.14	-0.11	-0.08	-0.07	-0.11	-0.02	-0.12	-0.10
	Episodes pH <5.8	Episodes pH <5.5	Episodes pH <5.2	DMI	Total VFA	Acetate	Propionate	A:P ratio	Butyrate				
Episodes pH <5.8	1												
Episodes pH <5.5	0.55**	1											
Episodes pH <5.2	0.20*	0.62**	1										
DMI (kg/d)	0.28**	0.18	0.11	1									
Total VFA (mM)	0.42**	0.16	0.05	0.39**	1								
Acetate (mmol/100 mol)	-0.63**	-0.61**	-0.24*	-0.44**	-0.35**	1							
Propionate (mmol/100 mol)	0.61**	0.63**	0.24*	0.40**	0.39*	-0.92**	1						
A:P ratio	-0.66**	-0.60**	-0.22*	-0.42**	-0.41**	0.94**	-0.98**	1					
Butyrate (mmol/100 mol)	0.05	-0.10	-0.08	0.04	-0.05	-0.15	-0.25*	0.13	1				

¹Min = minimum; Max = maximum; Mod = moderate; A:P ratio = acetate:propionate ratio.* $P < 0.05$; ** $P < 0.01$.

Table 4. Eigenvalues and the contribution of principal components (PC) to the total variance in ruminal fermentation and acidosis

Item	Eigenvalue	Proportion of total variance (%)	Cumulative variance (%)
PC1	11.5	52.2	52.2
PC2	4.19	19.0	71.2
PC3	1.22	5.53	76.7
ΣPC4 to PC22	5.12	23.3	100

Severity of RA, Period, and Interactions

Severity of RA, period, and their interaction for ruminal fermentation, severity of RA, intakes, and production variables are presented in Tables 6 and 7. Ruminal pH variables were lower postpartum than prepartum, with greater differences between periods for the MA group than the LA group (Table 6). A significant interaction was observed between severity of RA and period for total RA duration and area, mild RA duration and area, moderate RA duration and area, and acute RA duration with significant differences between periods for the MA cows and no difference between periods for the LA cows. Daily episodes for total RA, mild RA, and acute RA were greater postpartum than prepartum. However, daily episodes for moderate RA showed significant interaction between severity and period, with

significant difference between periods for the MA group and IN group and no difference between periods for the LA group. The RPS of *Prevotella* and *M. elsdenii* were not influenced by severity of RA, period, or the interaction between them (Table 6). Dry matter intake was greater postpartum than prepartum and was not different among the groups differing in severity of RA (Table 7). Severity of RA and period effects tended to be different for total VFA concentration. Ruminal propionate proportion was greater postpartum than prepartum and acetate proportion was lower postpartum than prepartum. Both ruminal acetate and propionate proportions were not different among the cows differing in severity of RA. None of the production variables were influenced by severity of RA (Table 7). The RPS of *Prevotella* and *M. elsdenii* were also not influenced by RA severity (Table 7).

DISCUSSION

Severity, Period, and Their Interactions

It is clear from Table 2 that considerable variability existed among cows in the severity of RA and that this variability was independent of the dietary treatment. Several studies have observed variation in severity of RA among cattle (Bevans et al., 2005; Penner et al., 2009b). Variations in severity of RA among cows can be

Table 5. Coefficients of the loadings (eigenvectors) and their contribution to the first 3 principal components (PC)

Variable ¹	PC1	Contribution for PC1 (%)	PC2	Contribution for PC2 (%)	PC3	Contribution for PC3 (%)
Ruminal pH						
Mean pH	−0.277	6.26	0.046	1.13	−0.024	0.71
Minimum pH	−0.254	5.75	0.080	1.97	−0.075	2.19
Maximum pH	−0.187	4.22	0.051	1.27	−0.173	5.04
Severity of RA						
Total RA duration (h/d)	0.283	6.41	−0.043	1.05	−0.165	4.81
Mild RA duration (h/d)	0.219	4.94	−0.212	5.25	−0.215	6.25
Moderate RA duration (h/d)	0.274	6.19	0.007	0.18	−0.227	6.60
Acute RA duration (h/d)	0.238	5.39	0.244	6.03	0.146	4.26
Duration pH <5.5 (h/d)	0.281	6.35	0.104	2.58	−0.093	2.70
Total RA area (pH × min)	0.162	3.66	0.303	7.49	0.049	1.43
Mild RA area (pH × min)	0.161	3.63	0.303	7.49	0.049	1.41
Moderate RA area (pH × min)	0.268	6.05	0.173	4.27	0.013	0.37
Acute RA area (pH × min)	0.198	4.47	0.262	6.47	0.247	7.19
Area pH <5.5 (pH × min)	0.261	5.89	0.204	5.06	0.077	2.25
Daily episodes pH <5.8	0.165	3.74	−0.281	6.95	0.004	0.12
Daily episodes pH <5.5	0.247	5.59	−0.107	2.64	−0.271	7.88
Daily episodes pH <5.2	0.242	5.46	0.180	4.45	0.070	2.03
Intake and rumen fermentation						
DMI (kg/d)	0.063	1.43	−0.220	5.44	0.522	15.20
Total VFA (mM)	0.078	1.76	−0.232	5.73	0.494	14.39
Acetate (mmol/100 mol)	−0.175	3.95	0.327	8.08	−0.052	1.51
Propionate (mmol/100 mol)	0.183	4.14	−0.322	7.98	−0.088	2.56
Acetate:propionate	−0.179	4.04	0.336	8.31	0.021	0.61
Butyrate (mmol/100 mol)	−0.030	0.67	−0.007	0.16	0.361	10.50
Total loadings (absolute)	4.424	100	4.042	100	3.437	100

¹RA = ruminal acidosis.

Figure 3. (a) Loading plot of principal component (PC) 1 and PC2 describing the relationships among ruminal fermentation parameters, various indicators of ruminal acidosis (RA), and DMI. Three clusters could be identified from the loading plot. The cluster in quadrant A consisted of ruminal pH variables [minimum pH (Min pH), mean pH, and maximum pH (Max pH)]. The cluster in quadrant B contained RA area (pH $\times$ min) variables [mild RA area (M area), moderate RA area (Mod area), acute RA area (A area), and total RA area (T area)], duration pH <5.5 (h/d), area under the pH curve <5.5 (area <5.5), and daily episodes of pH <5.2 (DE <5.2). The cluster in quadrant C consisted of RA duration (h/d) variables [mild RA duration (M duration), moderate RA duration (Mod duration), and total RA duration (T duration)], daily episodes of pH <5.8 and <5.5 (DE <5.8 and DE <5.5), and ruminal propionate (Prop; mmol/100 mol). Acet = ruminal acetate; Buty = ruminal butyrate; Acet:Prop = acetate:propionate ratio; n = 22 variables. (b) Loading plot of principal component PC1 and PC3 describing the relationships among ruminal fermentation parameters, various RA indicators, and DMI. Two clusters separated by PC1 could be identified from the loading plot. The cluster in the left quadrants consisted of ruminal pH variables (Min pH, mean pH, and Max pH), Acet (mmol/100 mol), and Acet:Prop. The cluster in the right quadrants contained all of the RA indicators [A area (pH $\times$ min); A duration (h/d); area <5.5 , DE <5.8 , <5.5 , and <5.2 ; T area; M area; duration pH <5.5 ; T duration; M duration; Mod duration; and Prop]. The variables DMI, total VFA (mM), and Buty were largely described by PC3. n = 22 variables.

Figure 4. Score plot of principal component (PC) 1 and PC2 by treatment (control or low-concentrate, Ctrl; high-concentrate, HC), describing the relationships among ruminal fermentation parameters, various indicators of ruminal acidosis, and DMI. Note that most of the data points corresponding to treatments did not show clustering by treatment, indicating a lack of treatment effect on the variables included in the model. n = 108.

Figure 5. Score plot of principal component (PC) 1 and PC2 by period, describing the relationships among ruminal fermentation parameters, various indicators of ruminal acidosis, and DMI. Note that most of the data points corresponding to the periods appear to cluster together for both control (Ctrl; a) and high-concentrate (HC; b) treatments. n = 54 for each treatment.

Figure 6. Relationship between the shifts in ruminal fermentation variables [principal components analysis (PCA) rank] and the shifts in ruminal bacterial communities [automated ribosomal intergenic spacer analysis (ARISA) rank] from the prepartum to postpartum period. Note that there was no relationship between PCA rank and ARISA rank ($n = 14$; $R^2 = 0.00$; $P = 0.98$).

caused by many factors, including differences in (1) the fractional rates of absorption of short-chain FA from the rumen, (2) osmotic pressure of ruminal fluid, (3) integrity and health of the ruminal epithelium (severity of parakeratosis), (4) genes regulating VFA absorption and metabolism, (5) VFA metabolism by the epithelium, (6) eating behavior, (7) salivation rate and ruminal fluid outflow, and (8) rumen microbial community composition. These factors can be broadly described as physiological, behavioral, and microbial differences among cows. Physiological differences affecting the susceptibility of sheep to RA upon induction were reported to occur due to differences in the rate of short-chain FA uptake (Penner et al., 2009a). In that study, SARA was induced using an oral drench of glucose and comparisons were made to water-drenched controls. Sheep exposed to the RA induction were further divided into responders and nonresponders based on the area under the curve of ruminal pH < 5.8 . They reported that the nonresponders had greater rates of uptake for acetate and butyrate than responders. Dohme et al. (2008), in a study evaluating the response to a repeated (in 3 periods) acidosis challenge in 2 risk groups (low risk and high risk) of dairy cows, reported individual animal differences in feeding behavior as well as in their response to the challenge. They observed that 1 out of the 4 cows in the high-risk group reduced its intake of the grain allotted during the acidosis challenge from periods 1 through 3 without eliminating the occurrence of RA, whereas another cow did not experience RA even though it consumed the entire grain allotted for each challenge. From the above discussion, it is clear

that physiological and behavioral differences among cows can alter their susceptibility to RA resulting in varied response in the severity of RA.

Despite the difference in severity of RA among individual cows, the differences in the RPS of *Prevotella* and *M. elsdenii* before and after calving were not significant, indicating that these bacteria may not be directly associated with RA. However, Khafipour et al. (2009) reported a decline in gram-negative *Bacteroidetes* organisms in dairy cattle induced with SARA (by replacing 21% of dietary DM with wheat-barley pellets), based on terminal restriction fragment length polymorphism analysis. The shift in the *Bacteroidetes* members (*Prevotella albensis*, *P. brevis*, and *Prevotella rumenicola*) in their study was also evident from the real-time PCR data. They also reported that *Streptococcus bovis* and *Escherichia coli* dominated in severe grain-induced SARA, whereas *M. elsdenii* dominated in mild grain-induced SARA. The lack of a difference in the RPS of *Prevotella* and *M. elsdenii* in the current study compared with that in the study of Khafipour et al. (2009) could be due to several reasons. The study of Khafipour et al. (2009) is different from the current study in that SARA was induced in their study, whereas the RA observed in the current study was acquired. When SARA is induced, the changes in ruminal fermentation variables are more acute compared with the gradual changes in fermentation in a naturally acquired RA. Furthermore, the cows in the study of Khafipour et al. (2009) were at the beginning ($\text{DIM} = 84 \pm 29$, mean $\pm$ SD; grain-induced SARA) and end of mid lactation ($\text{DIM} = 175 \pm 75$; alfalfa pellet induced SARA), unlike the transition cows in the current study. Differences in stage of lactation and diets offered between the 2 studies could possibly account for the differences observed in the RPS of *Prevotella* and *M. elsdenii*.

Several studies indicate that acidotic conditions in the rumen can lead to milk fat depression (Kalscheur et al., 1997; Shingfield et al., 2005; Mohammed et al., 2010) with a simultaneous increase in the RPS of ruminal *M. elsdenii* (Weimer et al., 2010b). Based on these findings, it was anticipated that milk fat percentage would be greater and RPS of *M. elsdenii* lower for the LA cows than MA cows. However, both milk fat percentage and RPS of *M. elsdenii* were not influenced by the variation in severity of RA (Table 7), although milk fat percentage ($P = 0.15$) and efficiency of milk yield ($P = 0.14$) tended to be lower. Thus, future research should explore the relationship between individual animal variation in severity of RA, milk fat percentage, and efficiency of milk yield.

The variation in severity of RA among cows was independent of DMI and total VFA concentration in rumen

Table 6. Effect of severity of ruminal acidosis (RA), period, and their interactions on ruminal pH, variables measuring the severity of RA, and proportions of ruminal bacteria

Variable	Least acidotic		Intermediate		Most acidotic		SEM	Significance		
	Pre ¹	Post ¹	Pre	Post	Pre	Post		Severity	Period	Severity × period
Ruminal pH										
Minimum pH	5.66	5.57	5.44	5.32	5.59	5.20	0.07	<0.01	<0.01	0.07
Mean pH	6.28	6.14	6.17	5.94	6.19	5.84	0.05	<0.01	<0.01	0.16
Maximum pH	6.90	6.71	6.86	6.57	6.75	6.55	0.05	0.02	<0.01	0.60
Severity of RA										
Total RA										
Daily episodes	5.17	7.86	7.16	12.1	5.34	13.1	1.33	0.06	<0.01	0.17
Duration (h/d)	2.49 ^c	4.07 ^c	3.57 ^c	8.07 ^b	2.59 ^c	10.9 ^a	1.06	0.01	<0.01	0.01
Area (pH × min)	174.1 ^b	944.0 ^b	513.9 ^b	940.6 ^b	334.2 ^b	5,689.3 ^a	732.7	<0.01	<0.01	<0.01
Mild RA										
Daily episodes	3.85	5.41	4.77	8.42	3.76	6.22	0.91	0.11	<0.01	0.54
Duration (h/d)	1.83 ^b	2.55 ^b	2.25 ^b	4.71 ^a	1.94 ^b	5.91 ^a	0.58	0.01	<0.01	0.03
Area (pH × min)	164.6 ^b	914.7 ^b	495.3 ^b	903.1 ^b	329.3 ^b	5,623.1 ^a	727.7	<0.01	<0.01	<0.01
Moderate RA										
Daily episodes	0.85 ^c	1.72 ^c	1.69 ^c	4.14 ^b	1.37 ^c	6.56 ^a	0.76	0.01	<0.01	0.02
Duration (h/d)	0.42 ^b	1.22 ^b	0.99 ^b	2.67 ^a	0.62 ^b	3.58 ^a	0.42	0.01	<0.01	0.04
Area (pH × min)	7.17 ^c	15.3 ^{bc}	12.8 ^{bc}	33.6 ^b	4.77 ^c	54.6 ^a	7.84	0.07	<0.01	0.03
Acute RA										
Daily episodes	0.47	0.76	0.70	1.50	0.22	2.60	0.53	0.31	0.01	0.13
Duration (h/d)	0.23 ^b	0.30 ^b	0.33 ^b	0.69 ^b	0.03 ^b	1.45 ^a	0.26	0.18	0.01	0.03
Area (pH × min)	2.28	3.05	5.77	3.88	0.05	11.6	2.93	0.53	0.16	0.07
RA index ² (pH × min/kg of DMI)	21.3 ^b	82.1 ^b	67.9 ^b	81.0 ^b	35.5 ^b	534.7 ^a	74.2	0.01	<0.01	0.01
RPS ³ of ruminal bacteria										
<i>Megasphaera elsdenii</i> (%)	0.03	0.03	0.06	0.03	0.03	0.27	0.09	0.45	0.41	0.33
<i>Prevotella</i> (%)	59.4	55.2	57.9	56.6	54.8	56.6	3.88	0.89	0.69	0.72

^{a-c}Least squares means within a row with different superscripts differ significantly ($P < 0.05$).

¹Pre = prepartum; Post = postpartum.

²RA index = area of pH <5.8, pH × min ÷ DMI, kg/d.

³RPS = relative population size; determined as the ratio of copies of the 16S rRNA gene of the target species to copies of the 16S rRNA gene amplified with universal bacterial primers and expressed as percentage.

Table 7. Effect of severity of ruminal acidosis, period, and their interactions on intakes, ruminal fermentation, and production parameters

Variable	Least acidotic		Intermediate		Most acidotic		Significance		
	Pre ¹	Post ¹	Pre	Post	Pre	Post	SEM	Severity	Period
Intake and ruminal fermentation variable									
DMI (kg/d)	11.7	15.4	10.6	15.0	11.5	14.2	0.70	0.54	<0.01
VFA (mM)	112.7	117.0	120.5	125.9	114.6	120.8	3.13	0.05	0.05
Acetate (mmol/100 mol)	66.4	62.4	65.1	59.8	66.3	60.2	0.84	0.10	<0.01
Propionate (mmol/100 mol)	18.8	22.2	19.5	25.4	18.2	24.8	0.86	0.11	<0.01
Butyrate (mmol/100 mol)	10.2	10.9	11.1	10.8	10.9	10.6	0.36	0.64	0.97
Acetate:propionate	3.57	2.93	3.38	2.44	3.67	2.56	0.15	0.11	<0.01
Production parameter									
Milk yield (kg/d)	—	27.7	—	31.2	—	30.6	1.72	0.32	—
Fat (%)	—	4.30	—	3.62	—	3.76	0.24	0.15	—
Fat yield (kg/d)	—	1.16	—	1.10	—	1.08	0.08	0.74	—
FCM (kg/d)	—	28.4	—	29.2	—	28.8	1.57	0.94	—
Milk yield/kg of DMI	—	1.82	—	2.08	—	2.16	0.11	0.14	—
FCM/kg of DMI	—	1.86	—	1.95	—	2.04	0.11	0.49	—

¹Pre = prepartum; Post = postpartum.

fluid (Table 7). However, an interaction was observed between severity of RA and period when total area that pH was <5.8 was normalized for DMI (reported as RA index; Table 6). The interaction between the main effects for RA index as well as for the other variables (total RA duration and area, mild RA duration and area, moderate RA duration and area, and acute RA duration) measuring severity of RA (Table 6) may be attributed to the dietary change at calving, differences in intakes between the prepartum and postpartum periods as well as to the differences in susceptibility to RA among the cows. Greater proportion of ruminal propionate and lesser acetate proportion in the postpartum period than in the prepartum period is attributed to the dietary change at calving and to the differences in intakes between periods.

It was one of our objectives to investigate if greater shifts in BCC from pre- to postpartum were associated with increased severity of RA in cows postpartum and, hence, an association between the ARISA grouping and the RA grouping would exist. However, only 2 of the 5 cows in each ARISA group (unstable or stable profiles) were also in the MA and LA groups, respectively (Table 2). The shifts in ruminal fermentation variables (PCA ranks) and the shifts in BCC (ARISA ranks) before and after calving were not related (Figure 6) suggesting that the shift in BCC observed across the periods was possibly not due to differences in the severity of RA. These findings are consistent with the study of Palmorari et al. (2010) in which it was reported that cows with different ruminal pH profiles had similar ruminal BCC.

Diet and Period Effects

It is clear from Figure 4 that RA was not influenced by diet, which confirms the results reported previously by Penner et al. (2007) with the same data set. However, a period effect on RA was evident for most of the cows based on the score plots of PC1 and PC2 (Figure 5a and 5b), also consistent with the findings of Penner et al. (2007). The period effect was expected based on the change in the dietary composition and DMI pre- and postpartum. It was anticipated that the BCC would be influenced by the dietary treatment due to the differences in the F:C ratios of the prepartum diets. Surprisingly, however, no effect of treatment was observed on BCC based on the lack of clustering of the ARISA profiles by treatment (Figure 1). Most studies that have reported differences in ruminal bacteria based on 16S rRNA sequencing used either a high-forage diet or a high-concentrate diet in their comparisons (Nagaraja and Titgemeyer, 2007; Fernando et al., 2010). In the current study, our objective was to investigate shifts in

BCC using diets suitable for transition cows. It is possible that the differences in F:C ratio of the diets used in the current study were not large enough to induce a measurable difference in BCC. A few studies (Steele et al., 2009; Chen et al., 2011) also reported shifts in epimural bacteria (attached to ruminal epithelium) when cows were transitioned from a high-forage to a high-grain diet. Although not explored in this study, we believe that the effect of RA on epimural bacteria is worth exploring because the microenvironment in the proximity of the ruminal epithelium has been shown to be different from that of the whole rumen with respect to VFA concentration and pH (Storm and Kristensen, 2010). It is not known if cows that are less acidotic in their first lactation remain so throughout their lifetime. Further studies are required to explore the persistency of severity of RA under different feeding conditions as well as at different times of the year.

CONCLUSIONS

This study investigated individual variability of dairy cows in their severity of RA during the transition period. Severity of RA (as assessed by duration of total RA, mild RA, moderate RA, and acute RA; area under the pH curve for total RA, mild RA, and moderate RA) increased postpartum for some cows, indicating variability in susceptibility to RA among cows. This variation was independent of intake, total VFA concentration, and individual VFA proportions. Production variables (milk yield, fat percentage, fat yield, FCM, and efficiency of milk production) were also not influenced by severity of RA. Some cows demonstrated shifts in bacterial communities across periods, but these cows were not necessarily the cows that were more acidotic. It can be concluded that not all cows were equally susceptible to RA and the shifts in BCC from pre- to postpartum were not related to differences in the severity of RA.

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