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Transient feeding of a concentrate-rich diet increases the severity of subacute ruminal acidosis in dairy cattle[1]

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ABSTRACT: The objective of this study was to investigate the effect of the pattern of concentrate-rich feeding on subacute ruminal acidosis (SARA), its severity, and the corresponding changes in VFA concentration. Eight rumen-cannulated Holstein cows were assigned to a 2 × 2 crossover design with 2 SARA challenge models and 2 experimental runs (n = 8 per treatment). Each run lasted for 40 d, consisting of a 6-d baseline, a 6-d gradual grain adaptation, and a 28-d SARA challenge period. The 2 SARA challenge models were transient (TRA) and persistent (PER) SARA. Initially, all cows were subjected to a forage-only diet (baseline) and gradually switched to 60% concentrate (DM basis). Then, cows in the PER model were continuously challenged for 28 d, whereas cows in the TRA model had a 7-d break from the SARA diet and were fed the forage-only diet after the first 7 d of SARA challenge. Thereafter, the TRA cows were rechallenged with the SARA diet. Wireless ruminal pH sensors were used to obtain ruminal pH profiles and temperature over the experimental period. For the determination of VFA, free ruminal liquid (FRL) and particle-associated ruminal liquid (PARL) were collected once for the baseline and twice (d 20 and 40 for the PER model) or 3 times (d 13, 30, and 40 for the TRA model) during SARA, each time at 0, 4, and 8 h after the morning feeding. Cows in both models experienced SARA albeit with day-to-day variation. From the start until the first 7-d SARA, cows of both models had similar pH profiles, but during the rechallenge, SARA was more severe in the TRA model than in the PER model based on lower daily mean ruminal pH (5.93 vs. 6.15; SEM 0.058) and double the amount of time at pH < 5.8 (497 vs. 278 min; SEM 68.61, $P < 0.05$). Mean ruminal temperature was raised during SARA compared with the baseline (38.9 vs. 38.7°C; SEM 0.057, $P < 0.001$). Concentrations of VFA increased with increasing time after feeding ($P < 0.001$). In general, SARA challenge (d 40 vs. the baseline), but not the challenge model, altered VFA concentrations and profile of both FRL and PARL by increasing the amounts of propionate and butyrate, whereas total VFA concentration was less affected. Proportions of VFA shifted over the duration of SARA challenge with more propionate but less acetate and butyrate proportions with advancing days of SARA challenge, leading to the values of the last SARA day being different from the earlier days ($P < 0.05$). In conclusion, the TRA condition led to the higher severity of SARA, but factors beyond feed intake and VFA alterations seemed to play a role.

Key words: cattle feeding, ruminal pH, subacute ruminal acidosis, volatile fatty acids

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INTRODUCTION

Feeding inadequate amounts of physically effective fiber increases the risk of subacute ruminal acidosis (**SARA**), which is characterized by intermittent and moderate drops of ruminal pH, for instance, at least 330 min/d at pH < 5.8 (Zebeli et al., 2008). Such a pH condition decreases ruminal digestion, especially of fiber, and impairs overall health and productivity (Plaizier et al., 2008). Indeed, severity of SARA and, therefore, its consequences for the digestion and health of the animal depends on feeding conditions. However, most of the studies on SARA assume permanent exposure to a SARA feeding challenge and these studies were mostly conducted during a relatively short period of induction time lasting from a few days to 1 or 2 wk (Dohme et al., 2008; Khafipour et al., 2009a,b). These conditions might not represent on-farm practices. For instance, obviously, in a dairy herd, cows experience SARA conditions that go far beyond 1 to 2 wk. Besides, SARA might be rather transient as animals temporally alter their intake pattern to restore ruminal balances (Palmonari et al., 2010; Gao and Oba, 2014). Once they return to the concentrate-rich diet, SARA might reoccur, possibly with higher severity (Dohme et al., 2008). However, long-term transient SARA conditions have been underexplored. Because ruminal microorganisms require time for their establishment in the rumen (Schwartzkopf-Genswein et al., 2003), a transient condition might lead to a greater challenge for ruminal microbes and the regulation of the intraruminal milieu. Accordingly, we hypothesized that a transient SARA results in greater severity of SARA in comparison with a persistent condition. This study investigated ruminal pH dynamics in 2 different long-term SARA challenge models, one transient and another persistent, with an attempt to relate these effects to VFA alterations.

MATERIALS AND METHODS

Animals

Eight rumen-cannulated (100 mm i.d.; Bar Diamond, Parma, ID) nonlactating Holstein cows (initial and final BW: 710 [SD 118] and 811 kg [SD 113], respectively; age: mean 68 mo [SD 20]) were used in the current experiment. During the experiment, the cows were kept together in a loose-housing stable with bedding at the Kremesberg research farm of Vetmeduni, Vienna. Animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to §26 of Law for Animal Experiments, Tierversuchsgesetz 2012-TVG (GZ 68.205/0093-II/3b/2013). To avoid confounding effects due to changes in the lactation phase and complications due to frequent rumen manipulations, we used nonlactating dairy cows.

Subacute Ruminal Acidosis Challenge Models and Feeding Methods

The 8 cows were blocked by BW and randomly assigned to a 2 × 2 crossover design (2 SARA models and 2 experimental runs), resulting in 8 observations per treatment. Each run consisted of a 40-d measurement period (a 6-d baseline, a 6-d gradual adaptation, and a 28-d SARA challenge; see below). There was a washout period of 8 wk in between the 2 runs to allow cows to recover from the high grain feeding. The 2 SARA challenge models were transient (**TRA**) and persistent (**PER**) SARA. The same diet with 60% concentrate (DM basis; the so-called SARA diet) but different feeding methods were applied to both SARA models. The ingredient and chemical composition of the diets (forage-only and SARA diets) used in the current study are presented in Table 1. At the start of the experiment, all cows were fed only the forage mix consisting of hay and grass silage (1:1 in DM) for 2 to 3 wk to adapt to the individual feeders. Subsequently, the cows were gradually adapted for 6 d to an increasing concentrate level at the rate of 10% per day to reach the target level at 60% (DM basis). Thereafter, cows in the PER model remained on the SARA diet continuously for 28 d. For cows in the TRA model, after gradual adaptation, SARA was induced for the next 7 d followed by a 7-d break from concentrate feeding in which the cows were fed the forage-only diet, the same as during the baseline. Thereafter, cows were rechallenged with the SARA diet for another 14 d (a 2-d gradual adaptation and a 12-d full challenge).

During the forage-only feeding (baseline and grain break) and until d 4 of the adaptation to grain, the diet was offered at 1.5% of BW, whereas starting from d 5 of grain adaptation and during the 60% grain feeding, DM intake was increased to 2.0% of BW. Forage mix and concentrate were offered separately in 4 and 2 feeding troughs, respectively, which were equipped with electronic weighing scales and computer-regulated access gates (RIC system; Insentec B.V., Marknesse, The Netherlands) to control distribution and the individual feed intake of each cow. To keep constant the forage and concentrate ratio of DMI, depending on the amount of daily forage consumption, the corresponding unconsumed concentrate, checked twice daily, was given through the cannula. Fresh feed was provided throughout the day. During the 4-wk SARA challenge, the concentrate amount needed to be delivered via the cannula was, on average, 2.48 ± 3.66 kg DM, accounting for approxi-

Table 1. Ingredients and chemical composition of weekly pooled forage-only diet and weekly pooled high-concentrate diet used for subacute ruminal acidosis (SARA) challenge

Item	Forage only[1]	SARA
Forage, % of DM		
Grass silage	50.0	20.0
Second-cut meadow hay	50.0	20.0
Concentrate, % of DM[2]		
Barley grain	0	19.8
Wheat	0	18.0
Corn	0	9.0
Rapeseed meal	0	10.2
Dried beet pulp	0	1.9
Calcium carbonate	0	0.3
NaCl	0	0.2
Mineral–vitamin premix[3]	0	0.6
Chemical composition, % of DM (unless otherwise stated)		
DM, %	54.4	74.5
OM	91.6	94.1
CP	12.8	15.4
NDF	51.7	31.8
ADF	36.2	19.9
Ether extract	1.50	1.71
Ash	8.36	5.86
NFC[4]	25.6	45.2

[1]During the baseline and concentrate break periods.

[2]Concentrate contained 88.0% DM, 95.8% OM, 17.2% CP, 1.9% ether extract, and 19.5% NDF (DM basis).

[3]Mineral–vitamin premix contained (per kg feed) 220 g Ca, 60 g P, 30 g Mg, 60 g Na, 3 g Zn, 5 g Mn, 0.01 g I, 0.04 g Se, 0.03 g Co, 0.75 g Cu, 600,000 IU vitamin A, 80,000 IU vitamin D, and 2 g vitamin E. Cows were offered free access to mineral licking stones (RINDAMIN LECKSTEIN; Schaumann GmbH & Co KG, Brunn, Austria) throughout the experiment.

[4]NFC = nonfiber carbohydrate: 100 – (ash – CP – NDF – ether extract).

mately 30% of the daily total concentrate intake. During the concentrate feeding periods, cows first had access to the forage mix starting from 0800 h whereas concentrate was offered 2 h later. Cows had continuous access to fresh water through a computer-regulated water trough (Insentec B.V.) and a salt licking stone. Daily intake data of feed and water were electronically measured.

Ruminal pH and Temperature Measurements

Wireless, remote-controlled ruminal pH sensors (smaXtec Animal Care, Graz, Austria) were used in this study to monitor ruminal pH and temperature dynamics, after a previous validation (Klevenhusen et al., 2014). The sensors were manually introduced via the rumen cannula in the baseline period. The data on ruminal pH and temperature on a real-time basis were continuously recorded every 10 min throughout the run. New sensors were used in the second run because the guaranteed performance of the sensors is limited to 50 d. Before use, the sensors were calibrated following the manufacturer's protocol. We characterized SARA when ruminal pH dropped below 5.8 for at least 330 min daily (Zebeli et al., 2008).

Ruminal Fluid Sampling and VFA Analysis

There were 2 types of ruminal fluid samples collected in the current study: one was free ruminal liquid (**FRL**) and another was particle-associated ruminal liquid (**PARL**), as previously described by Tafaj et al. (2004). Approximately 200 mL of FRL was initially collected from the ventral ruminal sac, a standard place for ruminal pH sampling (Zebeli et al., 2012), by inserting an aspiration tube (RUMINATOR; T. Geishauser, Guelph, Canada) through the rumen cannula. Then, approximately 200 g of solid digesta from the ruminal mat were manually taken and squeezed through 4 layers of cheesecloth to obtain PARL (available only in the second run). Samples of both rumen liquid fractions were collected at different phases and different hours (0, 4, and 8 h) after morning forage feeding. In the TRA model, samples were collected on d 5 (baseline), 13 (the first day of SARA challenge), 30 (the third day of rechallenge), and 40 (the last day of SARA challenge). The samples in the PER model were taken on d 5, 20, and 40. All samples were immediately stored at –20°C for further analyses.

Concentrations of individual VFA (acetate, propionate, isobutyrate, *n*-butyrate, isovalerate, *n*-valerate, and caproate) were determined by gas chromatography. Thawed FRL and PARL samples were centrifuged at 20,000 × *g* for 25 min at 4°C to remove solid materials. The clear supernatant (0.6 mL) was transferred into a fresh tube and 0.2 mL of HCl (1.8 mol/L) and 0.2 mL of internal standard (4-methylvalerian acid) were added. This mixture was again centrifuged at 20,000 × *g* for 20 min at 20°C to remove precipitated substrates. Subsequently, the supernatant was analyzed for VFA concentrations via gas chromatography (GC model 8060 MS DPFC, number 950713; Fisons, Rodena, Italy). The gas chromatograph was equipped with a flame-ionization detector and a 30 m by 0.530 mm by 0.53 μm capillary column (Trace TR Wax; Thermo Fisher Scientific, Waltham, MA). The injector and detector had temperatures of 170 and 190°C, respectively. Helium was used as carrier gas with a flow rate of 1 mL/min. Chromatograms were generated and evaluated using Stratos Software (Stratos version 4.5.0.0; Polymer Laboratories, Church Stretton, Shropshire, UK).

Chemical Composition Analysis of Feed

Feed samples were collected weekly and analyzed for DM, ash, OM, CP, ether extract, and NDF following the protocol for nutrient proximate analysis (VDLUFA,

2007), and subsequently, nonfiber carbohydrate was calculated [100 – (NDF + CP + ether extract + ash)]. Before analysis, samples were air-dried (54°C for 48 h), ground, and randomly sampled for further analyses. Dry matter was determined by oven drying at 100°C for 24 h. Samples were combusted at 580°C overnight for the determination of ash content. Crude protein was analyzed following the Kjeldal method (VDLUFA, 2007) and ether extract using a Soxhlet extraction system (Extraction System B-811; Büchi, Flawil, Switzerland). The NDF content of the diet was determined using Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany) with heat-stable α-amylase.

Statistical Analysis

All data was analyzed using PROC MIXED of SAS (version 9.2; SAS Inst. Inc., Cary, NC). For all analyses, differences between means were declared significant at $P < 0.05$ and a trend for significance at $0.05 \leq P < 0.10$. Data on daily intake, ruminal pH, and temperature during the course of 40 d of measurement were available for all cows. These data were analyzed with a statistical model following a crossover design (Cheng et al., 2005). Cows were coded into 2 sequences depending on the order of SARA model that they were subjected to. The feeding period was classified into 5 phases, which were the baseline, adaptation, SARA challenge, break, and SARA rechallenge. The fixed factors of the model included sequence, experimental run, SARA model, phase, and the interaction of SARA model and phase, considering the random effects of cows nested within sequence. The measurements within each phase taken on the same cow for several days were considered repeated measures in the model and a first-order autoregressive variance–covariance matrix was used to account for the repeated measures of individual cows. The linear mixed model is shown as follows:

$$Y_{ijklm} = \mu + R_i + T_j + S_k + P_l + TP_{jl} + C_{m(k)} + e_{ijklm},$$

in which Y_{ijklm} is the dependent variable, μ is the overall mean, R_i is the fixed effect of the ith experimental run, T_j is the fixed effect of the jth SARA model, S_k is the fixed effect of the kth sequence, P_l is the fixed effect of the lth phase, $C_{m(k)}$ is the random effect of the mth cow within the kth sequence, and e_{ijklm} is the residual error.

There were 2 statistical models for VFA data. For the first analysis, we used data at the baseline and on the last day of SARA, when samples of both SARA models were taken. After an initial validation indicating insignificant effects of the SARA challenge model, the data of both SARA models were pooled and then analyzed following the statistical model including sequence, experimental run, sampling time, phase (baseline vs. SARA), and the sampling time × phase interaction, and cows nested within experimental run were considered random effects as follows:

$$Y_{ijklm} = \mu + R_i + T_j + S_k + P_l + TP_{jl} + C_{m(i)} + e_{ijklm},$$

in which Y_{ijklm} is the dependent variable, μ is the overall mean, R_i is the fixed effect of the ith experimental run, T_j is the fixed effect of the jth sampling time, S_k is the fixed effect of the kth sequence, P_l is the fixed effect of the lth phase, $C_{m(i)}$ is the random effect of the mth cow within the ith experimental run, and e_{ijklm} is the residual error.

Another statistical analysis was used to examine VFA changes in more detail during the course of SARA (only for FRL samples). The 2 SARA challenge models were analyzed separately. For each challenge model, the data of all sampling days were used to evaluate the fixed effects of SARA phase (SARA days), time after morning feeding, and their interaction, and the baseline was defined as a covariate in the statistical model. The same random term as the previous analysis of VFA data was used in the model as follows:

$$Y_{ijkl} = \mu + S_i + T_j + ST_{ij} + b(X_{ijk} + \bar{x}) + C_{l(i)} + e_{ijkl},$$

in which Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of the ith SARA day, T_j is the fixed effect of the jth sampling time, X_{ijk} is the covariate of the ith SARA day and the jth sampling time, b is the regression coefficient, $\bar{x}$ is the mean of X, $C_{l(i)}$ is the random effect of the lth cow within the ith experimental run, and e_{ijkl} is the residual error.

RESULTS

Feed Intake

Means of total DMI, forage, and concentrate intake (expressed as kg DM/d) and water intake are shown in Table 2. Total DMI was affected by SARA model, phase, and the interaction between model and phase ($P < 0.001$). The intake remained similar between the 2 SARA models from the baseline until the first 7-d SARA challenge. During the break, as planned, cows in the TRA model were fed principally forage (10.4 kg DM/d), whereas cows in the PER model continued on the 60% concentrate diet (SARA diet) and, therefore, had lower intake of forage (5.90 kg/d; $P < 0.05$) but greater concentrate intake (9.14 kg/d; $P < 0.05$) compared with TRA cows. During rechallenge, the DMI and concentrate intake of PER cows were higher than that of TRA cows ($P < 0.05$). There was a phase effect ($P < 0.039$) for water intake with the highest water intake found during the adaptation period.

Table 2. Feed intake (kg DM/d) and water intake (kg/d) as affected by subacute ruminal acidosis (SARA)[1] challenge model and feeding phase[2]

	SARA model[3]							
	Phase							
Item	Day 1–6 (forage only)	Day 7–12 (adaptation)	Day 13–19 (SARA)	Day 20–26 (break for TRA)	Day 27–40 (SARA)	SEM	*P*-value	
							Model	Phase	Interaction
Total DMI						0.698	<0.001	<0.001	<0.001
TRA	11.03	13.76	14.88	10.71[b]	15.22[b]				
PER	11.42	13.74	15.72	15.06[a]	16.14[a]				
Forage intake						0.424	<0.001	<0.001	<0.001
TRA	10.59	8.22	5.93	10.42[a]	6.58				
PER	10.58	8.18	5.96	5.90[b]	6.44				
Concentrate intake						0.438	<0.001	<0.001	<0.001
TRA	0.44	5.52	8.89	0.30[b]	8.58[b]				
PER	0.85	5.54	9.76	9.14[a]	9.68[a]				
Water intake						4.449	0.099	0.039	0.660
TRA	47.72	51.34	48.13	43.48	48.47				
PER	48.16	50.85	51.22	47.92	49.96				

[a,b]For each variable, means of both SARA models in the same column differ ($P < 0.05$).

[1]Challenged using a high-concentrate feeding (60% of DMI).

[2]After 1 wk of SARA challenge (Day 13-19), there was a break for the transient model followed by SARA rechallenge (Day 27–40); cows in the persistent model had a continuous 28-d SARA challenge.

[3]TRA = transient; PER = persistent.

Ruminal pH Dynamics and Temperature

Data of daily ruminal pH and the time spent under certain ruminal pH thresholds (5.5, 5.8, and 6.0) as well as ruminal temperature variables are shown in Table 3. The SARA model affected only minimum and maximum ruminal pH variables ($P < 0.001$), whereas a phase effect was found with all pH variables ($P < 0.001$). In general, lower ruminal pH values and longer durations of ruminal pH below the thresholds were observed during the SARA challenge phases compared with the baseline (forage-only) values ($P < 0.05$). Importantly, except for time with pH < 5.5 ($P = 0.076$), the interaction between SARA model and phase was found to be significant for ruminal pH variables ($P < 0.01$), by which the differences between the 2 models were detected only during the break (except maximum pH) and thereafter. In addition, maximum pH was also higher in the PER model than in the TRA model during the first 7 d of SARA challenge ($P < 0.05$). In detail, daily mean ruminal pH of both SARA models dropped approximately 0.20 units starting from the first day of adaptation and continuously declined until reaching the mean pH of 6.10 during the first 7 d of SARA challenge (Table 3; Fig. 1). The durations of ruminal pH below 5.5, 5.8 and 6.0 increased when the concentrate diet was fed to the animals, as evident during the first 7 d of SARA challenge (Table 3; Fig. 2). During this phase, cows in both models had, similarly, approximately 200 to 300 min per day, a ruminal pH below 5.8 and, approximately 100 and 500 min per day, a ruminal pH below 5.5 and 6.0, respectively. The similar ruminal pH condition (approximately 6.16 mean pH and approximately 270 min per day at pH < 5.8) of cows in the PER model was sustained thereafter. For the TRA model, during the concentrate break, cows in this model had higher ruminal pH (mean and minimum) and shorter durations at ruminal pH below 5.8 and 6.0 than those in PER model ($P < 0.05$). Once rechallenged with the high-concentrate diet d 27-40, TRA cows showed a stronger severity of SARA compared with those of the PER model. During this phase, TRA cows had a lower mean ruminal pH (–0.22 units) and spent almost the double amount of time at all ruminal pH thresholds than the PER model ($P < 0.05$), but there was no difference in the minimum pH ($P > 0.05$).

Diurnal changes of ruminal pH on the last 3 d of the SARA challenge (d 38–40), an average of both runs, are illustrated in Fig. 3. A similar general pattern was found among the days that ruminal pH reached the maximum around the time of forage feeding. After concentrate feeding, the decline of ruminal pH began and the nadir was reached around 10 to 11 h after morning concentrate feeding. Despite the similar patterns, ruminal pH of the TRA model was approximately –0.3 units lower ($P < 0.05$) than that of the PER model most of the time, except for during 4 to 10 h after concentrate feeding when both models had similarly low pH values. The model difference was more evident on d 40 when ruminal pH of the TRA model was lower than that of the PER model throughout the day ($P < 0.05$).

Ruminal temperature variables (mean, minimum, and maximum) were affected by the SARA model ($P < 0.05$; maximum temperature, $P < 0.01$) and phase

Table 3. Ruminal pH and temperature as affected by subacute ruminal acidosis (SARA)[1] challenge model and feeding phase[2]

	SARA model[3]								
	Phase								
Item	Day 1–6 (forage only)	Day 7–12 (adaptation)	Day 13–19 (SARA)	Day 20–26 (break for TRA)	Day 27–40 (SARA)	SEM	*P*-value		
							Model	Phase	Interaction
Mean pH						0.058	0.604	<0.001	<0.001
TRA	6.38	6.11	6.06	6.37[a]	5.93[b]				
PER	6.38	6.16	6.08	6.18[b]	6.15[a]				
Minimum pH						0.072	0.010	<0.001	<0.001
TRA	6.09	5.66	5.47	6.11[a]	5.40				
PER	6.11	5.71	5.40	5.51[b]	5.48				
Maximum pH						0.049	<0.001	<0.001	<0.001
TRA	6.64	6.47	6.49[b]	6.62	6.35[b]				
PER	6.69	6.53	6.63[a]	6.63	6.64[a]				
Time pH < 5.5, min/d						41.48	0.947	0.003	0.076
TRA	21.1	51.3	111.7	6.40[x]	208.1[a]				
PER	21.1	47.9	121.3	109.7[y]	106.9[b]				
Time pH < 5.8, min/d						68.61	0.583	<0.001	0.003
TRA	31.8	225.7	220.6	26.4[b]	497.3[a]				
PER	30.9	181.6	317.6	267.4[a]	277.7[b]				
Time pH < 6.0, min/d						91.05	0.884	<0.001	0.003
TRA	84.8	465.4	546.4	96.5[b]	713.7[a]				
PER	43.8	395.5	499.4	416.9[a]	482.9[b]				
Mean temperature, °C						0.057	0.017	<0.001	0.002
TRA	38.75	38.72	38.84	38.71[b]	38.79[b]				
PER	38.69	38.72	38.79	38.88[a]	38.91[a]				
Minimum temperature, °C						0.456	0.030	0.829	0.186
TRA	33.70	32.96[b]	33.57	33.36	33.20[b]				
PER	33.31	33.94[a]	33.87	34.02	33.86[a]				
Maximum temperature, °C						0.070	0.003	<0.001	<0.001
TRA	39.46	39.52	39.64	39.41[b]	39.58[b]				
PER	39.42	39.50	39.63	39.75[a]	39.77[a]				

[a,b]For each variable means of both SARA models in the same column differ ($P < 0.05$).

[x,y]For each variable means of both SARA models in the same column tend to differ ($P < 0.10$).

[1]Challenged using a high-concentrate feeding (60% of DMI).

[2]After 1 wk of SARA challenge (Day 13–19), there was a break for the transient model followed by SARA rechallenge (Day 27–40); cows in the persistent model had a continuous 28-d SARA challenge.

[3]TRA = transient; PER = persistent.

(mean and maximum, $P < 0.001$) and there were interactions between model and phase for mean and maximum ruminal temperatures, meaning that the PER model had higher mean and maximum temperatures than the TRA model only during the break and during the rechallenge ($P < 0.05$; Table 3). However, the differences were not biologically significant (at maximum, +0.2°C). On average across phases and models, mean and maximum ruminal temperature were 38.8 and 39.8°C, respectively. The minimum temperature reached as low as 33.0°C (TRA; adaptation period).

Ruminal VFA

The VFA concentration and composition of both FRL and PARL during the baseline and SARA (d 40) are shown in Fig. 4 and Table 4, respectively. Similarly, in both ruminal samples, total and individual VFA (acetate, propionate, and butyrate) concentrations increased with increasing time after feeding ($P < 0.001$) and there was no interaction between SARA and time (Fig. 4). However, the effect of SARA on VFA concentrations in both ruminal sample types was not identical. At the start of the experiment (baseline), both FRL and PARL were similar in total VFA concentration, which was 100 m*M* at 8 h after feeding, consisting of acetate, propionate, and butyrate at the ratio of 67:20:10 (Fig. 4; Table 4). Compared with the baseline, SARA did not affect total VFA concentration of FRL, because acetate decreased at the expense of butyrate and propionate concentrations. Total VFA concentration of PARL, on the other hand, significantly increased during SARA (+30%, at 8 h) as a result of increased

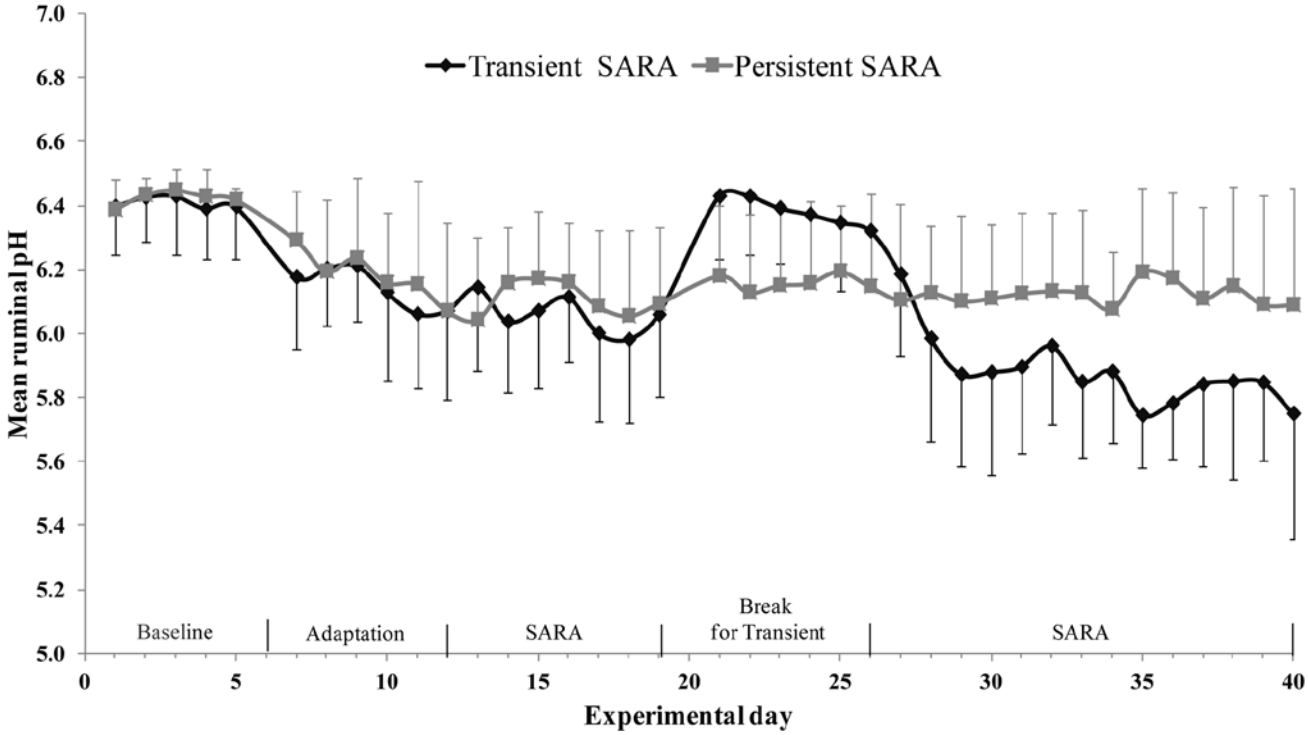

Figure 1. Ruminal pH profile during 40 experimental days as affected by subacute ruminal acidosis (SARA) challenge model (n = 8 per model per time point). Error bars represent SD. After 7 d of SARA challenge (d 13–19), there was a break (d 20–26) of grain feeding for the transient model followed by SARA rechallenge (d 27–40), whereas cows in the persistent model had a continuous 28-d SARA challenge (d 13–40).

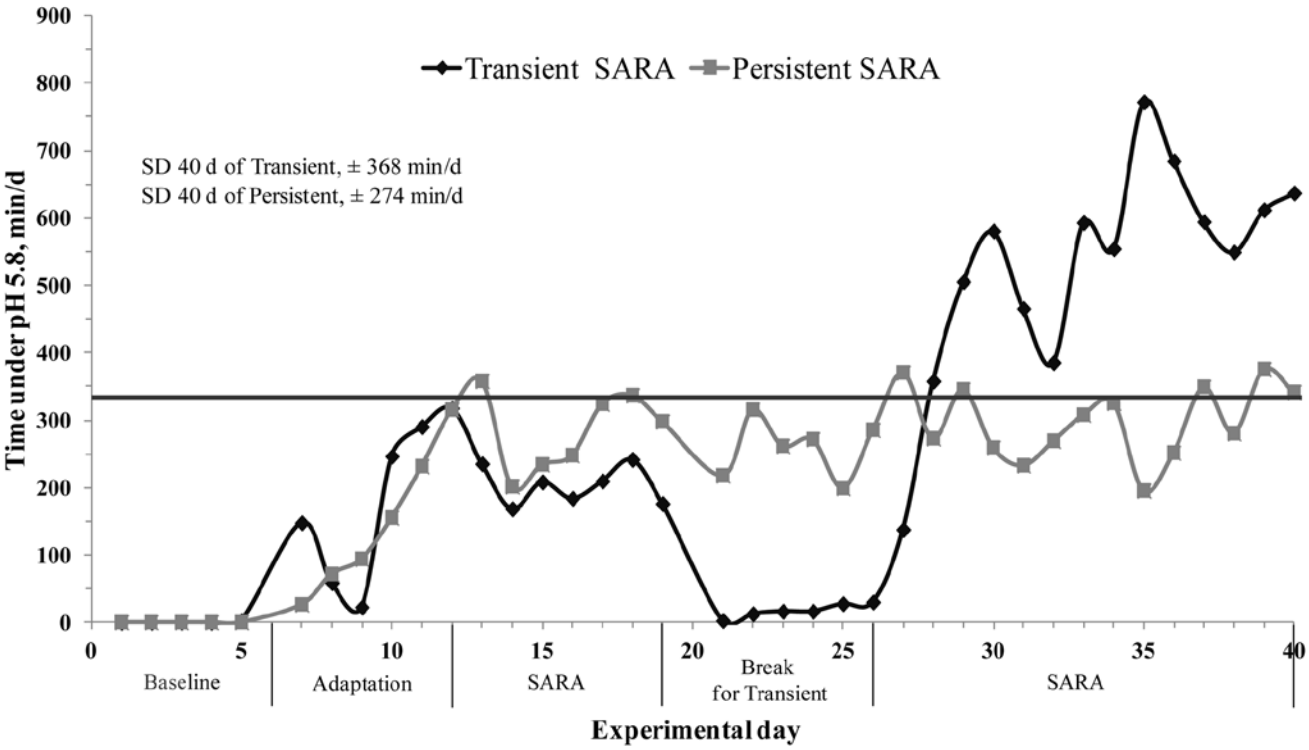

Figure 2. Average time spent under ruminal pH < 5.8 in 2 subacute ruminal acidosis (SARA) challenge models during 40 experimental days (n = 8 per model per time point). After 7 d of SARA challenge (d 13–19), there was a break (d 20–26) of grain feeding for the transient model followed by SARA rechallenge (d 27–40), whereas cows in the persistent model had a continuous 28-d SARA challenge (d 13–40). Thick bar indicates SARA condition (i.e., minimum 330 min/d at pH < 5.8) according to Zebeli et al. (2008).

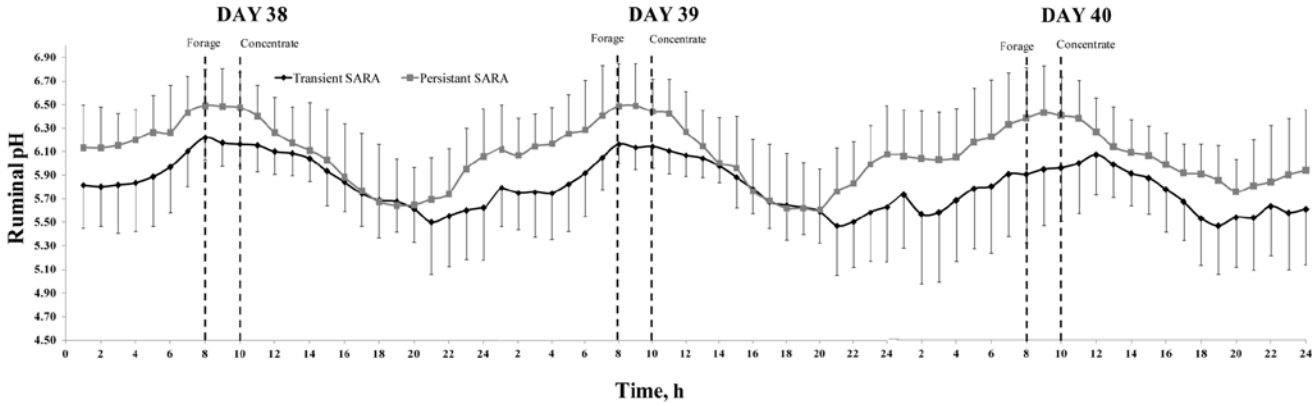

Figure 3. Diurnal change of ruminal pH on the last 3 d of subacute ruminal acidosis (SARA) challenge of the 2 different SARA challenge models (SARA model effect, $P < 0.05$; hour effect, $P < 0.001$; $n = 8$ per SARA model per time point). Error bars represent SD.

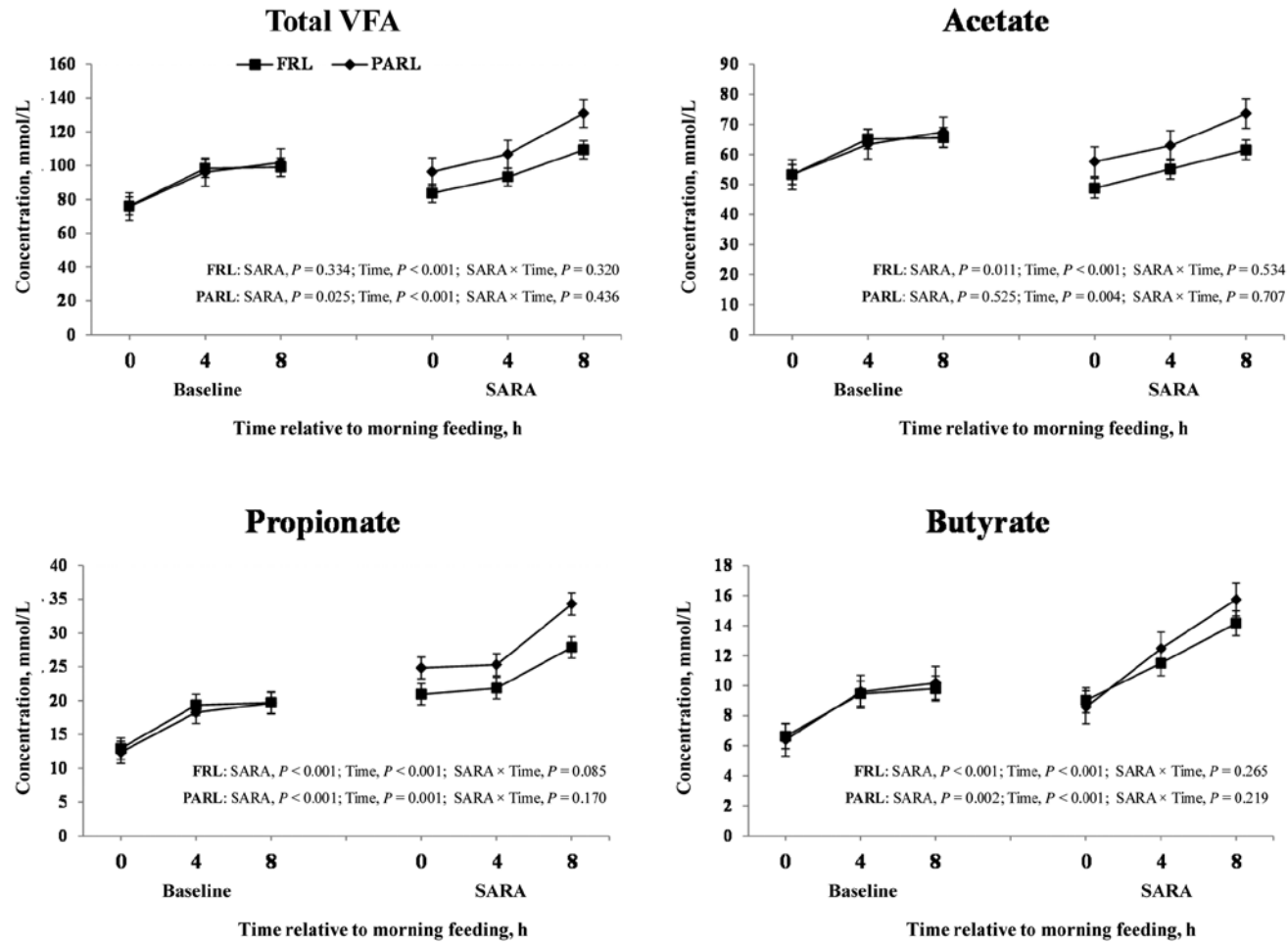

Figure 4. Changes in VFA concentrations (mmol/L) of free ruminal liquid (FRL) and particle-associated ruminal liquid (PARL) in relation to time after morning forage feeding during the forage-only diet (baseline, d 5) and concentrate challenge with 60% concentrate of diet DM (subacute ruminal acidosis [SARA], d 40). Data of the transient and persistent SARA models are pooled ($n = 8$ per period per time point). Error bars represent SEM.

Table 4. Volatile fatty acid composition (% of total VFA) of free ruminal liquid (FRL) and particle-associated ruminal liquid (PARL) as affected by subacute ruminal acidosis (SARA) challenge and time after morning feeding. Data of 2 SARA challenge models were pooled across each feeding phase

	Phase (experimental day)									
	Baseline (d 1)[1]			SARA (d 40)[2]						
	Time, h[3]							*P*-value		
Item	0	4	8	0	4	8	SEM	Phase	Hour	Phase × hour
FRL										
Acetate	70.34	66.69	66.37	58.85	59.11	56.30	1.031	<0.001	0.002	0.084
Propionate	16.67	19.37	19.78	24.73	23.67	25.53	0.922	<0.001	0.054	0.066
Butyrate	8.58	9.53	9.87	10.39	12.03	13.01	0.390	<0.001	<0.001	0.222
Isobutyrate	1.15	1.04	0.9	1.21	0.86	0.72	0.069	0.075	<0.001	0.148
Valerate	1.1	1.41	1.37	2.19	2.20	2.18	0.186	<0.010	0.569	0.584
Isovalerate	1.42	1.41	1.17	1.83	1.53	1.56	0.150	0.007	0.121	0.453
Caproate	0.683	0.548	0.545	0.798	0.584	0.69	0.099	0.181	0.141	0.818
Acetate-to-propionate ratio	4.28	3.49	3.40	2.65	2.57	2.28	0.179	<0.001	<0.001	0.092
PARL										
Acetate	70.50	66.57	66.68	60.59	59.34	56.18	1.867	<0.001	0.019	0.455
Propionate	16.26	18.66	19.08	25.33	23.77	26.48	1.620	0.001	0.108	0.132
Butyrate	8.42	9.83	9.84	8.76	11.54	12.06	0.456	<0.001	<0.001	0.121
Isobutyrate	1.28	1.14	0.99	0.98	0.81	0.70	0.066	<0.001	<0.001	0.942
Valerate	1.22	1.58	1.47	2.2	2.46	2.40	0.302	0.010	0.374	0.974
Isovalerate	1.61	1.59	1.35	1.57	1.33	1.43	0.179	0.765	0.136	0.222
Caproate	0.712	0.627	0.595	0.562	0.748	0.753	0.184	0.858	0.861	0.228
Acetate-to-propionate ratio	4.39	3.62	3.54	2.95	2.63	2.20	0.371	0.002	0.054	0.762

[1]All cows fed forage-only diet.

[2]Cows were challenged with a high-concentrate feeding (60% of DMI).

[3]Time after morning forage feeding (concentrate was fed 2 h after forage feeding).

propionate (+60%) and butyrate concentrations (+60%), whereas the acetate concentration remained unchanged in comparison with the values of the baseline.

Although VFA concentrations of FRL and PARL differed in response to SARA, the changes in their VFA composition were quite similar (Table 4). For both ruminal sample types, the molar percentage of acetate decreased whereas the molar percentages of propionate, butyrate, and valerate increased during SARA compared with the baseline. As a result, at 8 h after feeding, the ratio of acetate:propionate:butyrate was approximately 56:26:13 for FRL and 56:26:12 for PARL. Minor variations were found with the iso-acids. Subacute ruminal acidosis increased isovalerate percentage but only of FRL, and SARA decreased the isobutyrate percentage in PARL. The time after feeding influenced the composition of several VFA ($P < 0.05$). Molar percentages of acetate and isobutyrate decreased, whereas butyrate and (only a trend) propionate percentages increased with increasing time after feeding. There was no interaction between SARA and time for the VFA composition.

With respect to changes in the VFA concentration and profile during the course of SARA development alone (baseline as covariable), only the data of total VFA concentration and the composition of the major VFA of FRL are presented (Fig. 5). Although total VFA concentration stayed constant during the entire SARA challenge in the PER model, there was a significant decrease in the percentage of acetate at the expense of propionate at the later SARA phase compared with the values of 20 d earlier ($P < 0.05$). Also, for the TRA model, the VFA profile of the last SARA phase differed from those of 2 previous samplings with a similar pattern of change in VFA profile toward propionate. In addition, butyrate was lower than during the previous days. Total VFA concentration during the last SARA phase was lower than at the beginning of challenge ($P < 0.05$).

DISCUSSION

Evaluating the Subacute Ruminal Acidosis Severity with Rumen Wireless Sensors

Close monitoring of the rumen environment is essential for interpreting animal responses. Ruminal pH is a crucial determinant for SARA; however, single or spot pH measurements are not accurate enough (Zebeli et al., 2008; Aschenbach et al., 2011). Therefore, any consideration of ruminal pH should include the pH range during the feeding cycle (Palmonari et al., 2010) and especially when rumen parameters can be measured in real time without disturbances to the animal (Krause and Oetzel,

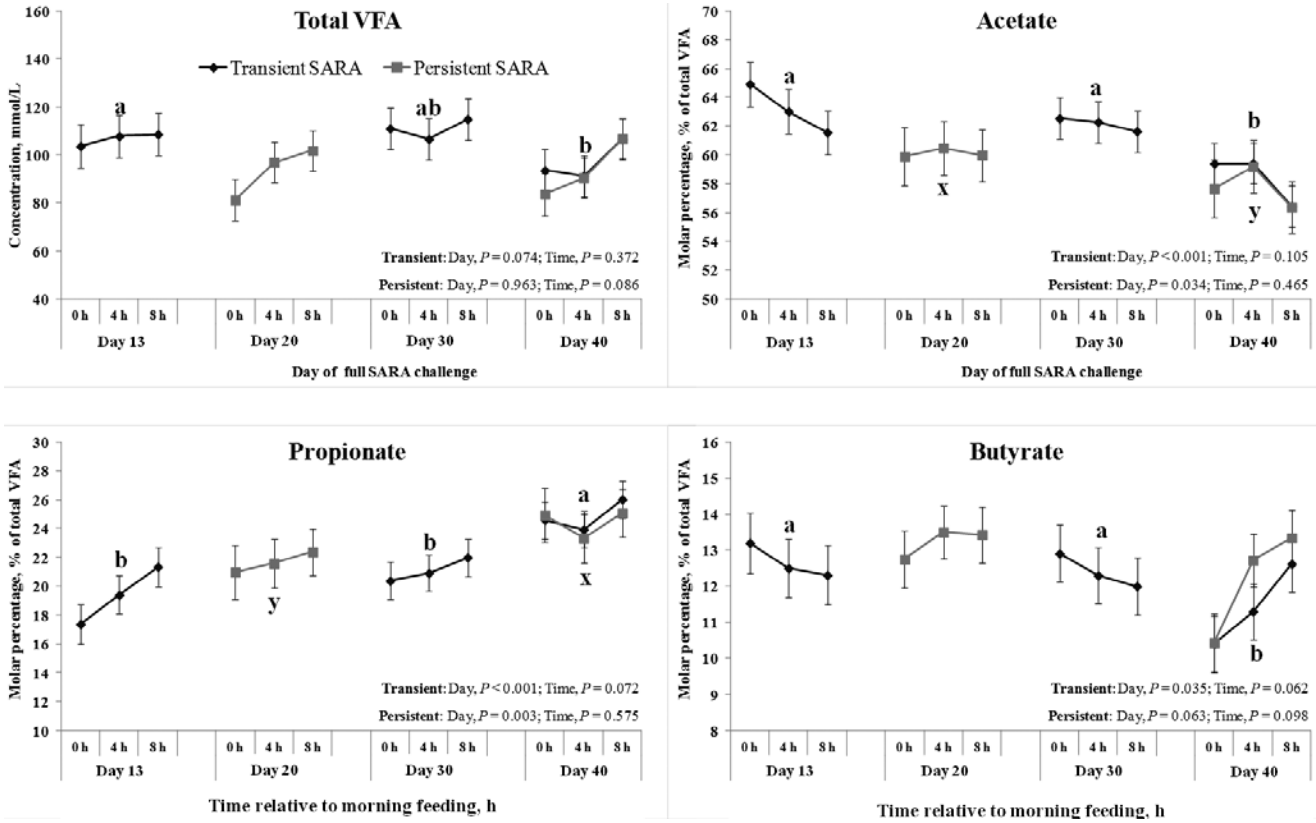

Figure 5. Changes in VFA concentration (mmol/L) and profile (% of total VFA) of free rumen liquid in relation to time after morning forage feeding during the course of the 28-d subacute ruminal acidosis (SARA) challenge period. Data of the challenge models were separately analyzed, and for each analysis, values of the baseline were treated as a covariate. Superscripts indicate the differences ($P < 0.05$) between the days of either the transient SARA model (a,b) or the persistent SARA model (x,y). Day 13 and 40 was the first and the last SARA challenge day, respectively. After 7 d of SARA challenge, there was a 7-d concentrate break for the transient model followed by SARA rechallenge, whereas cows in the persistent model had a continuous 28-d SARA challenge. There was no interaction between day of SARA challenge and time after feeding for all VFA variables ($P > 0.05$). n = 8 per treatment per time point. Error bars represent SEM.

2006). Accordingly, rumen wireless sensors are, so far, a useful tool for ruminant nutrition studies (Klevenhusen et al., 2014; Castro-Costa et al., 2015). Our companion study (Klevenhusen et al., 2014) revealed that the wireless sensors can satisfactorily reflect the pH of FRL, the common type of rumen samples for pH measurement. We diagnosed SARA when ruminal pH was <5.8 for at least 330 min per day (Zebeli et al., 2008). We also evaluated SARA with other pH thresholds such as 5.5 and 6.0 (Krause and Oetzel, 2006). In the current study, rumen sensors recorded ruminal pH and temperature every 10 min and the resulting data permitted the more accurate evaluation of SARA condition and its severity as not only pH threshold values but also the duration of ruminal pH below the thresholds was measured. It can be seen that when relying on daily mean pH values alone, the PER model showed no SARA condition at all, because the mean pH remained above 6.00 for the entire challenge period (Fig. 1), and SARA occurred in cows in the TRA model only a couple of days during the rechallenge phase. However, when considering the duration of ruminal pH < 5.8, cows in both models did experience SARA with day-to-day variations. Moreover, the SARA was more severe in cows in the TRA model, having lower ruminal pH and longer duration of low ruminal pH (Fig. 1 and 2). Consistent with previous recommendations (Zebeli et al., 2008; Palmonari et al., 2010; Aschenbach et al., 2011), our results again emphasize the major influence of the pH measurement method for the evaluation of SARA.

Subacute Ruminal Acidosis Challenge Model Affecting the Severity of Subacute Ruminal Acidosis

An interesting finding of this study was that SARA was more severe in the TRA model than in the PER model and that this severity was pronounced only in the rechallenge phase. Until the first phase of SARA challenge (d 13-19) both models performed very similarly regarding ruminal pH profiles. But after the grain break, cows in the TRA model experienced almost twice the amount of time at all SARA thresholds studied (pH < 5.5, pH < 5.8, and pH < 6.0) than cows in the PER model. This difference was certainly not caused by greater grain intake because cows in the PER model had slightly higher DMI and concentrate intake than the ones in the TRA model. In agreement with our results, Dohme et al. (2008), who

investigated 3 repeated single SARA challenges (1-d induction) with 4 kg of barley and wheat grain in lactating cows, observed that the severity of SARA increased with repeated exposure. They underlined the avoidance of cows toward the grain allocation, which increased with the repeated challenge as a mechanism to correct ruminal imbalances, although this did not always attenuate the effects of SARA. Our results and those of Dohme et al. (2008) indicate that there are factors beyond supply of rapidly fermentable carbohydrates that can influence the severity of SARA in dairy cattle.

Changes in the VFA balance (i.e., production and neutralization processes) affect ruminal pH (Allen, 1997; Dijkstra et al., 2012). A decreased ruminal pH can be expected in the scenarios of increasing total VFA in the rumen from increased production and decreased absorption of VFA (Dijkstra et al., 2012). However, our VFA concentration and composition results can explain only the effect of SARA that the fermentation favored propionate and butyrate production at the expense of acetate (Fernando et al., 2010) but not the different severity of SARA between the TRA and PER models. This could have been partly associated with the sampling time. Our last sampling was done at 8 h after concentrate feeding, in which cows of both models displayed quite similar ruminal pH (Fig. 3), although we did not find big differences on 0 h after morning feeding either. It might be more important to measure the samples obtained shortly after the nadir was reached, assuming the peak fermentation and accumulation of the resulting fermentation acids.

Nevertheless, a closer evaluation of the diurnal ruminal pH changes in response to feeding hours (Fig. 3) revealed that absorption of VFA of the PER model might be higher than that of the TRA model, particularly during the resting time. Despite the steeper decline of ruminal pH, reaching low ruminal pH similar to those of the TRA model after concentrate was offered, ruminal pH of the PER model rose back up to the higher levels during the night, reflecting a greater VFA absorption. The persisting SARA condition might give the rumen papillae enough time to adapt to increase the absorptive capacity. The ruminal epithelial adaptation was probably related to functional adaptations rather than morphological changes to maximize the absorptive area, which may require a period of 6 to 8 wk (reviewed by Khiaosaard and Zebeli, 2014). However, other morphological changes that may also influence VFA absorption such as thinning of rumen epithelial layer and disruption of tight junctions, which increase permeability and paracellular transport, was found to occur 1 wk after feeding a high-grain diet (Steele et al., 2011). An increase in ruminal VFA absorption will also increase secretion of endogenous bicarbonate from the rumen epithelium, another significant means of buffering capacity (Dijkstra et al., 2012). It is still unclear whether ruminal feed digestion and, therefore, overall VFA production differed between the 2 models, but the presumed increase in VFA absorption could likely decrease the severity of SARA of PER animals. Likewise, Dohme et al. (2008) also presumed a decrease in VFA absorption to be responsible for the increasing severity of SARA with repeated grain challenges, based on the results of a previous study (Krehbiel et al., 1995) showing that short-term ruminal acidosis decreased VFA absorption in the rumen. Nevertheless, it is noteworthy that contribution of VFA absorption to the susceptibility of SARA is still inconclusive. Observations regarding VFA absorption in response to SARA insults varied considerably among studies (Thorlacius and Lodge, 1973; Penner et al., 2009b; Schwaiger et al., 2013) and among cows within the same study (Penner et al., 2009a; Gao and Oba, 2014). As is known, the metabolic status of animals plays an important role in rumen digestion and absorption. For example, the adaptive response of the rumen to an increased VFA load was more pronounced during the periparturient period (Bannink et al., 2012). In nonlactating and nonpregnant cows, ruminal VFA absorption might not even be affected by increased VFA production in the rumen (Penner et al., 2009b). Still, our results suggested that a substantial change in ruminal VFA absorption can be detected in nonpregnant cows when the SARA challenge duration is extended.

Ruminal microorganisms require time for their establishment in the rumen (Schwartzkopf-Genswein et al., 2003). Fluctuations of ruminal pH due to inconsistent supply of nutrients will have a direct consequence on microbial balances in the rumen. Our results indicate that maintaining an altered ruminal environment over a prolonged period might be more favorable for ruminal microbiota. The fermentation profile toward propionate production during SARA observed in the present study indicated a rise of amylolytic species and a decrease of cellulolytic species (Fernando et al., 2010) in both SARA challenge models. But it is plausible that with sufficient time of exposure, there were "stable" orchestrated changes to facilitating the balances between the new community, and that may lessen the severity of SARA in the PER model.

Ruminal pH, and, therefore, severity of SARA, is regulated by not only the supply of substrates for ruminal microbes and removal of VFA (ruminal absorption and passage) but also neutralization of VFA via bicarbonate secretion from saliva and the rumen epithelium and exogenous buffering of feedstuffs (Dijkstra et al., 2012). The amount of saliva secretion depends on feed intake level and dietary fiber as well as animal activity (Maekawa et al., 2002; Dijkstra et al., 2012). As the same diet was used for SARA challenge of the 2 models, there-

fore, variations in activity (e.g., eating, ruminating, and resting) and meal pattern, which can affect saliva secretion, among cows in both SARA models may have to the severity of SARA. Recent data in beef cattle (Schwaiger et al., 2013) showed that advancing time of exposure to a high-concentrate diet helped to stabilize ruminal pH (decreased day-to-day variation) and this seemed to be related to changes in meal pattern, buffering strategies, and ruminal acid production. According to Gao and Oba (2014), cows tolerant to a high-concentrate diet seemed to sort and chew less than intolerant ones.

Other relevant factors for an accurate diagnosis of SARA are related to pH measurement such as the frequency, the location of the rumen, and the type of ruminal sample (Palmonari et al., 2010; Zebeli et al., 2012; Klevenhusen et al., 2014). In line with that, another important remark gained in this current study is the difference of VFA results between FRL and PARL. Both ruminal fluid fractions had similar VFA concentrations during the baseline, but the effect of SARA challenge on total VFA concentration seemed to be more evident in PARL as a result of the difference of SARA effect on acetate concentration. Acetate concentrations of PARL during the baseline and SARA did not differ, whereas SARA decreased the acetate concentration of FRL. Fiber degradation takes place primarily within the ruminal mat because of a higher density of substrates, including retained undigested and potentially degradable particles (Klevenhusen et al., 2014), and adherent cellulolytic bacteria (Tafaj et al., 2004). Similar to the current finding, Tafaj et al. (2004) reported a higher VFA concentration, especially of acetate, in the dorsal rumen compared with the ventral part. It is clear that VFA do not equally distribute within the rumen content, and our results underline that this can be more evident under SARA condition.

In the present experiment, 2 SARA models were successfully induced using a 60% concentrate diet, when taking into account the amount of time at low pH. Initially, cows in both models performed similarly, but after the 1-wk break was imposed, the TRA condition increased the severity of SARA. The fermentation profile of FRL shifted toward more propionate and butyrate and less acetate during SARA, but total VFA concentration was unaffected. However, the effect of SARA on VFA concentrations of PARL was not identical to that of FRL, as acetate was not decreased and, therefore, total VFA increased during SARA, marking possible concomitant effects of the sample types. Accordingly, site of sampling should be considered for future studies under the scope of ruminal acidosis. The difference in the severity of SARA between the 2 SARA challenge models cannot be explained by VFA. Ruminal absorption of VFA, microbial adaptation and compensation, animal activity, and buffering strategy presumably contributed to the susceptibility of SARA. The 2-d adaptation before the SARA2 challenge may also have played a role in the susceptibility of SARA. Elucidation of the order of importance of these factors and evaluations of consequences of TRA concentrate feeding for ruminal fermentation and cow health warrant further research. Also, further research is needed to understand the mechanisms behind this greater severity of SARA due to TRA challenge and also to determine its consequences for rumen health, nutrient degradation, and systemic health.

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