Effect of a grain challenge on ruminal, urine, and fecal pH, apparent total-tract starch digestibility, and milk composition of Holstein and Jersey cows

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ABSTRACT

The effects of a grain challenge on ruminal, urine, and fecal pH, apparent total-tract starch digestibility, and milk composition were determined. Six Holstein cows, 6 rumen-cannulated Holstein cows, and 6 Jersey cows were used in a replicated 3 × 3 Latin square design balanced to measure carryover effects. Periods (10 d) were divided into 4 stages (S): S1, d 1 to 3, served as baseline with regular total mixed ration ad libitum; S2, d 4, served as restricted feeding, with cows offered 50% of the total mixed ration fed on S1 (dry matter basis); S3, d 5, a grain challenge was performed, in which cows were fed total mixed ration ad libitum and not fed (CON) or fed an addition of 10% (MG) or 20% (HG) pellet wheat-barley (1:1) top-dressed onto the total mixed ration, based on dry matter intake obtained in S1; S4, d 6 to 10, served as recovery stage with regular total mixed ration fed ad libitum. Overall, cows had a quadratic treatment effect for milk yield where CON (22.6 kg/d) and HG (23.5 kg/d) had lower milk yield than cows in MG (23.7 kg/d). Jersey cows had a quadratic treatment effect for dry matter intake where cows in CON (13.2 kg/d) and HG (12.4 kg/d) had lower dry matter intake than cows in MG (14 kg/d). Holstein cows had a linear treatment effect for dry matter intake (17.7, 18.4, and 18.6 kg/d for CON, MG, and HG, respectively). Rumen pH for the rumen-cannulated cows had a linear treatment effect (6.45, 6.35, and 6.24 for CON, MG, and HG, respectively). Cows in HG spent more time with rumen pH below 5.8 (4.33 h) than MG (2 h) or CON (2.17 h) as shown by the quadratic treatment effect. Holstein cows in HG (8.46) had lower urine pH than MG (8.51) or CON (8.54) as showed by the linear treatment effect for urine pH. Apparent total-tract starch digestibility had a tendency for a linear treatment effect on S3 (97.62 ± 1.5, 97.47 ± 1.5, and 91.84 ± 1.6%, for CON, MG, and HG, respectively). Fecal pH was associated with rumen

pH depression as early as 15 h after feeding for Holstein cows. In conclusion, a grain challenge reduced urine pH in Holstein cows but not in Jersey cows. Holstein cows' health were not affected when rumen pH was depressed. A potentially useful link between rumen pH and systemic (urine) pH within 2 h after feeding was quantified in Holstein cows.

Key words: grain challenge, acidosis, pH, starch digestibility

INTRODUCTION

Dairy cows transitioning from the dry to the lactating period, and feedlot cattle, have to rapidly adapt from a high-forage to a high-concentrate diet, causing apparent changes in the ruminal environment. The introduction of rapidly fermentable carbohydrate to meet the cows' increased requirement for energy reduces fibrolytic bacteria, increases amylolytic bacteria, and decreases ruminal pH (Tajima et al., 2001). An abrupt change from a high-forage to a high-concentrate diet can result in acute or subacute acidosis (Coe et al., 1999). Acute acidosis establishes as a noticeable illness (Owens et al., 1998), but subacute acidosis is more difficult to recognize. Subacute ruminal acidosis is a common condition affecting dairy cows and has up to 26% incidence in commercial dairy farms (Garrett et al., 1999; Kleen et al., 2003). Its association with DMI depression, milk yield depression, reduced feed efficiency, rumenitis, diarrhea, laminitis, inflammation, liver abscesses, and high culling and death rates in dairy cattle has been extensively reported by previous authors (Kleen et al., 2003; AlZahal et al., 2007; Kmicikewycz and Heinrichs, 2014).

It is still unclear how a grain overload or SARA affects feed digestibility throughout the digestive tract. Subacute ruminal acidosis can decrease rumen NDF digestibility and affect fecal consistency (Krajcarski-Hunt et al., 2002). Krajcarski-Hunt et al. (2002) reported that SARA reduced 24 and 48 h in situ NDF digestibility of forages by an average of 20 and 25%, respectively. Similar results were reported by Plaizier et al. (2000). Feces of cows experiencing SARA had

Received April 5, 2015.

Accepted December 2, 2015.

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a greater DM content, longer particles, whole grains, sweet-sour smell, and appeared to be brighter, yellowish, liquid (Oetzel, 2000; Kleen et al., 2003; Li et al., 2012). The alterations might be caused by postruminal fermentation in the intestines due to a massive outflow of fermentable carbohydrates from the rumen, or binding of fluid in the intestinal lumen with high osmolality (Oetzel, 2000). This suggests that the ruminal microbial population and passage rate may be affected by SARA.

Nocek and Tamminga (1991) reported estimates for rumen starch digestibility based on the fractional degradation rate from in situ studies. These authors concluded that the variability of starch digestibility was high and that the amount of starch consumed had little effect on defining ruminal and total-tract starch degradation for adult dairy cattle using in vivo data digestibility. Firkins et al. (2001) summarized in vivo rumen starch digestibility in dairy cattle, as well as the effect that processing methods had on both total-tract starch digestion and milk production response to high-starch feeds. The authors concluded that lactating cows fed diets with low ruminal degradability of starch had higher, almost complete compensatory digestion of starch in the hindgut, thereby resulting in relatively minor improvements in total-tract OM digestibility or lactation performance for the more highly available starch sources, assuming similar DMI.

It has long been understood that rumen pH depression is one of the factors associated with decreased milk fat percentage along with high rumen unsaturated fatty acid load (Byers and Schelling, 1988; Boerman et al., 2014). A grain challenge leading to SARA can facilitate incomplete biohydrogenation of fatty acids and increased *trans*-octadecenoic acids, and especially *trans*-10 isomer of *trans*-octadecenoic acid, which is associated with milk fat depression (Byers and Schelling, 1988). It is still not well understood how a grain challenge affects cow milk production and milk protein synthesis. Decreased milk production caused by SARA has been reported previously (Stone, 2004; Colman et al., 2013). Stone (2004) observed a decrease in milk protein yield. However, other authors (Fairfield et al., 2007; Khafipour et al., 2009; Colman et al., 2013) have reported increased milk protein yield. Plaizier et al. (2008) suggested that the change in rumen digestible OM from microbial protein synthesis might play a role on the inconsistent results for milk protein yield. The literature and the aforementioned observations were based on research using either Holstein cows or beef steers. The effects of a grain challenge on milk yield and protein yield production of Jersey cows are unclear. It is known that milk fat composition of Holsteins differs from Jerseys, and that short-chain fatty acids are the major source of milk fatty acid (Drackley et

al., 2001). Effects of a grain challenge on urine and fecal pH have been reported by others (Enemark, 2008; Morgante et al., 2009; Giancesella et al., 2010); however, different time points and frequency of sample collection make them difficult to aggregate. Understanding how rumen, urine, and fecal pH are associated after a grain challenge in Jersey and Holstein cows, and its effect on production parameters, deserves attention. Therefore, the objectives of this experiment were (1) to determine the effects of a grain challenge on ruminal, urine, and fecal pH, apparent total-tract starch digestibility, and milk composition of lactating cows; and (2) determine potential differences in response to a grain challenge by lactating Holstein and Jersey cows.

MATERIALS AND METHODS

Animals and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee. The experimental period occurred during the months of October and November 2012. Cows were housed in tiestalls with sand bedding and ad libitum water and feed access. Diet (TMR) was formulated according to NRC (2001) recommendations.

Experimental Design and Grain Challenge Procedure

Eighteen animals were selected and assigned into 1 of 3 groups. The first group had 6 Holstein cows (**HOL**), parity 2.67 ± 1.5 , BW 717 ± 30 kg, and 258 ± 16 DIM; the second group had 6 rumen-cannulated Holstein cows (**CAN**), parity 3.86 ± 1.0 , BW 774 ± 30 kg, and 287 ± 45 DIM; and the third group had 6 Jersey cows (**JER**), parity 2.50 ± 1.5 , BW 471 ± 30 kg, and 190 ± 86 DIM. This experiment was conducted as a replicated 3×3 Latin square design balanced to measure carryover effects. Therefore, treatments were arranged so that the carryover effects could be evaluated.

The grain challenge was similar to the one used for SARA induction proposed by Krause and Oetzel (2005). Briefly, each cow received all treatments in different periods. Each period had 10 d and was divided into 4 stages. The first stage (**S1**), d 1 to 3, served as baseline with regular TMR ad libitum. The second stage (**S2**), d 4, served as restricted feeding, with cows offered 50% of the TMR fed on S1 (DM basis). In the third stage (**S3**), d 5, a grain challenge was performed, in which cows were fed TMR ad libitum and not fed (0%) or fed an addition of 10 or 20% pellet wheat-barley (1:1), based on DMI obtained in S1. Pellets were top-dressed onto the TMR and the consumption by the

Table 1. Ingredient composition of the lactation diet fed to cows in control (CON), moderate (MG), and high (HG) treatments throughout the experimental period

Ingredient	% of DM
Alfalfa hay	3.35
Grass hay	2.80
Corn silage	33.60
Alfalfa silage	9.31
Whole cottonseed	8.01
Soy hulls	4.64
Dry ground corn grain	21.0
Lactating supplement ¹	17.2

¹Lactating supplement was formulated for 43.6% CP, 13.4% NDF, 7.1% ADF, 0.3% lignin, 5.47% crude fat, and 25.21 mEq/100g DCAD, and contained: 24.7% soybean meal, 26.16% bypass protein (SoyPLUS, West Central, Ralston, IA), 1.94% bypass fat (Energy Booster 100, Milk Specialties Global, Paris, IL), 7.53% blood meal, 4.3% sodium bicarbonate, 6.13% limestone, 2.26% dicalcium phosphate, 1.18% white salt, and <1% of each of the following: trace minerals, vitamin A, D, and E.

cows was observed visually. The fourth stage (**S4**), d 6 to 10, served as recovery stage with regular TMR fed ad libitum. Daily DMI was measured with a 10% refusal target. Feed was delivered (0800 h) and orts weighed individually for each cow.

Experimental treatments were control (**CON**), no addition of wheat-barley (1:1) pellet; moderate grain

challenge (**MG**), 10% of S1 DMI as wheat-barley (1:1) pellet top-dressed; and high grain challenge (**HG**), 20% of S1 DMI as wheat-barley (1:1) pellet top-dressed. Cows were fed the same TMR during all the aforementioned stages and had it delivered once a day.

Data Collection and Sampling Procedures

Samples of feed ingredients and TMR (Tables 1 and 2) were obtained in the first day of each period and analyzed for DM contents (AOAC International, 2000) by drying for 24 h in a forced-air oven at 110°C. Diet DM was adjusted for changes in DM content of ingredients. Total mixed ration samples (a sample of TMR delivered to each cow, $n = 18$) were taken on d 3 of each period and stored at -20°C until analyzed. Composite samples (1 per period) were analyzed for contents of DM, CP, ADF, NDF, lignin, starch, fat, ash, NFC, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S using wet chemistry methods (Dairy One, Ithaca, NY; <http://dairyone.com/wp-content/uploads/2014/02/Forage-Lab-Analytical-Procedures.pdf>). Value for NE_L was provided by the laboratory and calculated based on NRC (2001). The physical characteristic of the TMR, based on the Penn State Particle Separator (Kononoff et al., 2003), was performed on d 3 of each period.

Table 2. Mean chemical composition (in % of DM unless otherwise indicated) and associated standard deviations for diets fed to cows in control (CON), moderate (MG), and high (HG) treatments throughout the 3 periods of the experiment

Item	Period ¹			SD
	1	2	3	
DM, %	47.1	47.9	47.1	0.57
CP	18.0	17.6	18.3	0.27
ADF	22.7	24.4	23.2	1.05
NDF	35.6	37.3	36.3	1.71
Lignin	4.53	4.00	3.80	0.29
NFC	33.4	32.6	33.2	1.71
Starch	22.3	21.4	23.6	1.73
Crude fat	5.23	4.70	4.57	0.19
Ash	7.79	7.77	7.59	0.25
NE_L , Mcal/kg of DM	1.63	1.63	1.65	0.04
Ca	0.98	1.07	1.02	0.10
P	0.44	0.45	0.45	0.01
Mg	0.28	0.29	0.31	0.01
K	1.51	1.55	1.41	0.05
Na	0.30	0.38	0.32	0.01
S	0.24	0.25	0.27	0.01
Fe	493	955	480	231
Zn	156	210	191	18.3
Cu	27.0	38.0	33.3	3.01
Mn	120	138	133	5.39
Mo	0.83	0.87	0.77	0.10

¹Each period had 4 stages: baseline (S1, d 1 to 3, cows fed normal TMR with ad libitum intake), restricted feeding (S2, d 4, cows fed 50% of the DMI in stage 1), grain challenge (S3, d 5, cows fed TMR + treatment), and recovery (S4, d 6 to 10), cows fed normal TMR with ad libitum intake. Diet was formulated for a Holstein lactating cow, BW of 650 kg, BCS of 3.5, milk of 40 kg/d, 3.5% milk fat, 3.2% milk true protein to provide MP of 2.6 kg/d and ME of 61.8 Mcal/d.

Rumen pH in CAN was measured every hour from -2 to 21 h after the grain challenge on S3. Representative samples of rumen fluid (40 mL) were collected and composited from 3 sites (ventral sac, cranial sac, and caudo-ventral blind sac) through the rumen cannula using a syphon. The pH of the fluid was measured with a portable pH meter (AP110 Fisher Scientific, Pittsburgh, PA) immediately after collection from the rumen.

Urinary catheters (Bardex Foley Catheter, REF 0166L22; C. R. Bard Inc., Covington, GA) were placed in the cow's bladder on d 4. All cows ($n = 18$) were catheterized. Briefly, a 15×10 cm rectangular area centered on the perineum was clipped and scrubbed with 15% Novalsan solution. The catheter was directed to the urethral orifice by putting a finger into the urethra, so that the catheter could follow the direction to the bladder. Sterile water (75 mL for Holstein cows and 65 mL for Jersey cows) was injected to the catheter balloon to keep it inflated during the collection period. To make sure enough sample volume was collected, the catheter was blocked 0.5 h before sampling. The catheter was removed by extracting the sterile water out of the balloon after the 24 h sampling period. Urine pH was measured every h from -2 to 21 h after the grain challenge on S3 in CAN, HOL, and JER.

Fecal samples (approximately 400 g, wet weight) were collected from the rectum of each cow into plastic containers at 7 time points (TP): TP1, d 1, 1500 h; TP2, d 5, 0900 h; TP3, d 5, 1500 h; TP4, d 5, 2200 h; TP5, d 6, 0700 h; TP6, d 6, 1500 h; and TP7, d 7, 1500 h. Fecal pH was measured immediately after the collection with portable pH meter (AP110 Fisher Scientific, Pittsburgh, PA), directly into the samples that were then stored at -20°C until analyzed. Fecal samples from TP1 to TP5 were analyzed for DM, starch, and lignin contents (Rock River Lab, Watertown, WI; Schalla et al., 2012). Lignin was used as marker to calculate the apparent total-tract starch digestibility (Church, 1988).

Cows were milked 3 times per day at 0600, 1400, and 2100 h. Milk weights were recorded daily and samples were obtained from consecutive milkings at am, pm, and night milking on d 2, 4, 5, and 8 of each period. Milk samples were composited by day in proportion to milk yield at each sampling and preserved (800 Broad Spectrum Mirotabs II; D&F Control Systems Inc., San Ramon, CA). Composite milk samples were analyzed for fat, true protein, lactose, urea N, total solids, and SCC using mid-infrared procedures (AOAC International, 1995) at a commercial laboratory (Dairy Lab Services, Dubuque, IA).

Health conditions recorded including body temperature, heart rate, respiratory score, general appearance,

rumination, and fecal score were evaluated daily and every 4 h on S3. Normal body temperature was considered elevated if $>39.4^{\circ}\text{C}$, heart rate was considered elevated if >100 beats/min, respiratory rate was considered abnormal if >40 breaths/min, general appearance was considered abnormal if ≥ 2 , where 1 = bright and alert, 2 = depressed, and 3 = reluctant to rise; rumen contractions (using a stethoscope) were considered abnormal if no movement was observed in a period of 5 min; and fecal score was considered abnormal if ≤ 2 (Ireland-Perry and Stallings, 1993; Krause and Oetzel, 2005).

Body weight was measured (Ohaus digital scale, model CW-11, Newark, NJ) and BCS was assigned in quarter unit increments (Ferguson et al., 1994) for each cow at S4 in each period. Three individuals assigned BCS independently at each time of scoring throughout the experiment and the average value was used.

Statistical Analyses

The data were analyzed using the mixed model procedure of SAS (v 9.2; SAS Institute Inc., Cary, NC) to account for carryover effect by the following model:

$$y_{ijklm} = \mu + S_i + A_{(i)j} + P_{(i)k} + T_l + C_m + e_{(ijk)l},$$

where y_{ijklm} is the observations for dependent variables; μ is the general mean; S_i is the fixed effect of the i th treatment sequence; $A_{(i)j}$ is the random effect of the j th cow in the i th sequence; $P_{(i)k}$ is the fixed effect of the k th period; T_l is the fixed effect of the l th treatment; C_m is the fixed carry-over effect from the previous period ($C = 0$, if period = 1); and $e_{(ijk)l}$ is the random error. If carryover effects were not detected, data were analyzed as a replicated Latin square by the following model:

$$y_{ijklm} = \mu + S_i + A_{(i)j} + P_{(i)k} + T_l + D_m + T \times D_{lm} + e_{(ijk)lm},$$

where y_{ijklm} is the observations for dependent variables; μ is the general mean; S_i is the random effect of the i th square; $A_{(i)j}$ is the random effect of the j th cow in the i th square; $P_{(i)k}$ is the fixed effect of the k th period; T_l is the fixed effect of the l th treatment; D_m is the fixed effect of repeated measurement, which used as TP in pH and day in DMI analysis; $T \times D_{lm}$ is the interaction of treatment and repeated measurement, and the interaction was removed if $P > 0.3$; and $e_{(ijk)lm}$ is the random error. The estimation method was REML, and the degrees of freedom method was Kenward-Roger (Littell

et al., 2002). Variables were subjected to 5 covariance structures: compound symmetry, autoregressive order 1, autoregressive heterogeneous order 1, unstructured, and Toeplitz. The covariance structure that yielded the lowest corrected Akaike information criterion was compound symmetry and used in the model (Littell et al., 2002). Milk SCC data were $\log_{10}$ transformed. Treatment linear and quadratic orthogonal contrasts were tested using the CONTRAST statement of SAS. Values reported are least squares means and associated standard errors of the mean. Area under the curve was calculated based on the incremental area method (Cardoso et al., 2011) with pH = 5.8 as the baseline. Residual distribution was evaluated for normality and homoscedasticity. The REG procedure of SAS was used to assess the association between rumen pH and urine pH on CAN. Statistical significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

RESULTS

The ingredient composition of the diets is detailed in Table 1. Analyzed nutrients from the experimental diet are shown in Table 2. The physical characteristic of the TMR, based on the Penn State Particle Separator (Kononoff et al., 2003), was $8.0 \pm 3\%$ on upper, $44.5 \pm 3\%$ on middle, $36.0 \pm 1\%$ on lower, and $11.0 \pm 2\%$ on bottom sieves. The carryover effect was tested and was not present for any variable of interest ($P > 0.30$).

DMI, BW, and BCS

Dry matter intake and DMI as a percentage of BW had an overall quadratic treatment effect ($P = 0.03$ and $P = 0.04$, respectively) where cows in CON and HG had lower DMI and DMI as a percentage of BW than cows in MG (Table 3). The overall data ($n = 18$) are composed of Holstein ($n = 12$) and JER ($n = 6$) cows, so the effect was across breeds. No treatment by stage interaction ($P = 0.13$) was observed even though numerically DMI was lower in S2 (Table 3). However, a tendency ($P = 0.07$) was observed for a treatment by stage interaction for DMI as percentage of BW; it was lower in S2.

Jersey cows had a quadratic treatment effect ($P < 0.01$) for DMI and DMI as a percentage of BW ($P < 0.01$) where cows in CON and HG had lower DMI and DMI as a percentage of BW than cows in MG (Table 3). Holstein cows had a positive linear treatment effect for DMI ($P = 0.04$) and DMI as a percentage of BW ($P = 0.02$). Body weight (654 ± 30 kg) and BCS (3.11 ± 0.3) were not different ($P = 0.3$) among treatments, and no treatment by period interaction was observed.

Lactation Performance

Milk yield had an overall quadratic treatment effect ($P = 0.02$) and had a positive linear treatment effect in HOL. Milk yield had a stage by treatment interaction ($P = 0.03$) for HOL and a tendency ($P = 0.08$) for the same interaction in JER (Table 3). Holstein cows in CON produced less milk than MG and HG cows on S4; however, milk yield were similar among treatments from S1 to S3. Overall ($n = 18$), the same effect was seen for FCM and fat yield (Table 3). Differences most likely happened because MG and HG received the pellets and therefore milk yield could be better maintained than CON in HOL cows. The same hypothesis could be made overall ($n = 18$) for FCM and fat yield. Treatments were not different for milk composition (Table 3). Milk SCC tended ($P = 0.08$) to have a negative linear treatment effect. Overall, 3.5% FCM and fat yield had a stage by treatment interaction (Table 3). Milk fat percentage and yield and 3.5% FCM had a tendency for a stage by treatment interaction in HOL (Table 3). No differences were observed for body temperature, heart rate, respiratory score, general appearance, rumination, or fecal score among treatments ($P > 0.10$).

Rumen, Urine, and Fecal pH

For visualization, the relationship among rumen, urine, and fecal pH over time for HG is shown in Figure 1. Rumen pH for the rumen-cannulated cows ($n = 6$) had a negative linear treatment effect ($P < 0.01$; Table 4; Figure 2). Cows in HG spent more time with rumen pH below 5.8 (4.33 h) as shown by the quadratic treatment effect ($P < 0.01$; Table 4). Overall, cows in HG had lower urine pH than MG or CON as shown by the negative linear treatment effect ($P = 0.01$) for urine pH. Interestingly, JER and HOL had different urine pH responses to the grain challenge. Differences among treatments for urine pH were not observed in JER but were observed for HOL cows (Table 4). Holstein cows had a tendency ($P = 0.09$) toward a negative linear treatment effect on fecal pH. Urine and rumen pH were associated for a narrow period (-2 to $+2$ h after feeding, $P = 0.05$; Figure 3) by the following formula: rumen pH = (urine pH - 6.2916)/0.2946; $R^2 = 0.77$, $P = 0.049$.

Apparent Total-Tract Starch Digestibility

Apparent total-tract starch digestibility had a tendency ($P = 0.06$) for a linear treatment effect on S3 ($97.62 \pm 1.5\%$, $97.47 \pm 1.5\%$, and $91.84 \pm 1.6\%$ for CON, MG, and HG, respectively). All cows had lower

Table 3. Least squares means and associated standard errors for DMI and milk parameters response to a grain challenge for cows in control (CON), moderate (MG), and high (HG) treatments at different stages

Item	Treatment ¹				Stage ²				P-value ³				
	CON	MG	HG	SEM	S1	S2	S3	S4	SEM	Stage × Trt	Period	Linear	Quadratic
Overall (n = 18)													
DMI, kg/d	16.2	16.9	16.4	0.53	16.1	7.07	18.3	16.7	0.79	0.13	0.41	0.09	0.03
DMI, % of BW	2.39	2.49	2.37	0.10	2.64	1.22	2.95	2.72	0.11	0.07	0.01	0.19	0.04
Milk yield, kg/d	22.6	23.7	23.5	1.89	23.5	22.9	18.6	22.1	0.48	0.24	<0.01	0.09	0.02
3.5% FCM, kg/d	24.0	24.7	24.6	0.95	25.3	24.6	20.9	24.5	1.90	0.04	0.01	0.35	0.43
Fat, %	4.12	3.96	4.14	3.96	4.30	4.13	4.32	4.13	0.14	0.12	0.53	0.74	0.98
Fat, kg/d	0.88	0.90	0.90	0.69	0.94	0.91	0.79	0.90	0.07	0.05	0.10	0.60	0.67
Protein, %	3.28	3.28	3.28	0.09	3.38	3.38	3.33	3.40	0.06	0.93	0.06	0.89	0.96
Protein, kg/d	0.72	0.75	0.74	0.35	0.76	0.76	0.61	0.76	0.05	0.13	0.11	0.21	0.31
Lactose, %	4.40	4.40	4.41	0.85	4.41	4.45	4.37	4.37	0.12	0.38	<0.01	0.87	0.96
Lactose, kg/d	1.01	1.05	1.04	0.09	1.05	1.04	0.83	1.02	0.10	0.14	<0.01	0.37	0.35
MUN, mg/dL	13.7	13.8	13.6	0.38	13.5	14.4	15.0	12.4	0.42	0.82	<0.01	0.62	0.43
SCC, × 1,000/mL	122	109	96.5	1.23	105	85.6	126	105	1.27	0.41	0.04	0.08	0.97
Jersey (n = 6)													
DMI, kg/d	13.2	14.0	12.4	0.63	13.9	6.16	14.7	13.8	0.79	0.64	0.11	0.70	<0.01
DMI, % of BW	2.51	2.82	2.44	0.18	2.94	1.31	3.13	2.99	0.21	0.63	0.30	0.58	<0.01
Milk yield, kg/d	18.7	19.1	19.4	2.37	19.8	20.0	15.2	20.8	3.77	0.08	0.22	0.30	0.13
3.5% FCM, kg/d	22.2	21.8	22.2	3.26	23.5	22.7	18.4	23.8	3.00	0.22	0.31	0.98	0.58
Fat, %	4.93	4.57	4.75	0.24	4.86	4.57	4.81	4.74	0.25	0.79	0.19	0.37	0.12
Fat, kg/d	0.88	0.84	0.86	0.07	0.93	0.86	0.73	0.93	0.09	0.59	0.16	0.68	0.32
Protein, %	3.61	3.69	3.65	0.09	3.68	3.67	3.58	3.67	0.11	0.75	<0.01	0.50	0.17
Protein, kg/d	0.65	0.70	0.68	0.02	0.71	0.73	0.54	0.73	0.09	0.16	0.51	0.22	0.19
Lactose, %	4.39	4.44	4.36	0.15	4.43	4.43	4.32	4.41	0.18	0.17	<0.01	0.64	0.16
Lactose, kg/d	0.82	0.86	0.85	0.12	0.88	0.91	0.67	0.91	0.14	0.13	0.34	0.41	0.43
MUN, mg/dL	14.0	14.5	13.9	0.48	13.3	14.5	15.7	12.9	0.47	0.72	<0.01	0.73	0.14
SCC, × 1,000/mL	4.64	4.42	4.51	0.25	97.5	79.04	120	78.3	1.35	0.93	0.01	0.47	0.30
Holstein (n = 12)													
DMI, kg/d	17.7	18.4	18.6	0.80	18.4	8.73	21.3	19.3	1.25	0.18	0.11	0.04	0.53
DMI, % of BW	2.18	2.32	2.34	0.12	2.48	1.18	2.85	2.60	0.13	0.04	<0.01	0.02	0.32
Milk yield, kg/d	24.6	25.9	25.6	2.03	26.9	25.9	21.9	25.1	2.29	0.03	<0.01	0.17	0.09
3.5% FCM, kg/d	24.9	26.1	25.7	1.81	27.1	26.4	23.0	25.8	0.29	0.09	0.06	0.34	0.27
Fat, %	3.63	3.78	3.66	0.15	3.75	3.65	3.81	3.56	0.17	0.08	0.79	0.87	0.30
Fat, kg/d	0.88	0.93	0.91	0.08	0.96	0.94	0.83	0.90	0.05	0.08	0.36	0.42	0.31
Protein, %	3.11	3.07	3.10	0.08	3.09	3.09	3.06	3.12	0.08	0.93	0.67	0.79	0.41
Protein, kg/d	0.75	0.77	0.77	0.05	0.82	0.79	0.67	0.79	0.06	0.17	0.01	0.44	0.66
Lactose, %	4.41	4.39	4.43	0.11	4.40	4.46	4.40	4.36	0.10	0.53	<0.01	0.69	0.54
Lactose, kg/d	1.10	1.14	1.13	0.19	1.02	1.17	0.98	1.14	0.07	0.17	<0.01	0.53	0.49
SCC, × 1,000/mL	4.88	4.82	4.60	0.30	116	94.6	137	128	1.39	0.49	0.39	0.12	0.60
MUN, mg/dL	13.6	13.5	13.5	0.52	13.4	14.2	14.6	12.0	0.55	0.76	<0.01	0.71	0.99

¹Treatment = dietary treatments were control diet (CON, with 0% of top-dress), moderate diet (MG, with 10% of top-dress), and high diet (HG, with 20% of top-dress).²Each period had 4 stages: baseline (S1, d 1 to 3, cows fed normal TMR with ad libitum intake), restricted feeding (S2, d 4, cows fed 50% of the DMI in stage 1), grain challenge (S3, d 5, cows fed TMR + treatment), and recovery (S4, d 6 to 10, cows fed normal TMR with ad libitum intake).³Contrasts were linear = linear treatment effect; and quadratic = quadratic treatment effect.

Table 4. Least squares means and associated standard errors for rumen, urine, and fecal pH response to a grain challenge for Holstein and Jersey cows in control (CON), moderate (MG), and high (HG) treatments

Item	Treatment ²			SEM	P-value ³	
	CON	MG	HG		Linear	Quadratic
Rumen ¹						
Mean pH	6.45	6.35	6.24	0.07	<0.01	0.85
Hour <5.8, h/d	2.17	2.00	4.33	0.78	<0.01	<0.01
Area under the curve, ⁴ pH × h/d	0.60	0.38	0.54	0.23	0.85	0.48
Mean urine pH						
Overall	8.48	8.46	8.43	0.02	0.01	0.63
Jersey	8.35	8.37	8.37	0.03	0.61	0.79
Holstein	8.54	8.51	8.46	0.24	<0.01	0.75
Mean fecal pH						
Overall	6.61	6.54	6.56	0.05	0.25	0.30
Jersey	6.69	6.68	6.73	0.06	0.70	0.68
Holstein	6.57	6.46	6.46	0.06	0.09	0.33

¹Rumen pH measures were taken only for the rumen-cannulated group.

²Treatment = dietary treatments were control diet (CON, with no top-dress), moderate diet (MG, with 10% of top-dress), and high diet (HG, with 20% of top-dress).

³Contrasts were linear = linear treatment effect; and quadratic = quadratic treatment effect.

⁴Area below pH threshold of 5.8.

($P = 0.02$) apparent total-tract starch digestibility at TP4 ($93.7 \pm 1.2\%$) when compared with TP2 ($97.4 \pm 1.2\%$), TP3 ($95.3 \pm 1.2\%$), and TP5 ($94.8 \pm 1.2\%$).

DISCUSSION

The aims of this study were to determine the effects of a grain challenge on ruminal, urine, and fecal pH, apparent total-tract starch digestibility, and milk composition, and to determine potential differences in response to a grain challenge by lactating multiparous Holstein and Jersey cows. We postulated that the grain

challenge might affect the rumen or intestinal environment or modulate the acid-base metabolism, thereby affecting DMI, nutrient supply, metabolic responses, and performance differently in Jersey and Holstein cows.

Grain challenge induced a reduction in rumen pH to lower than 5.8 for the HG treatment, indicating that cows could have experienced a certain degree of SARA (Penner et al., 2007). Others were more successful in reducing rumen pH after a grain challenge (Krause and Oetzel, 2005; Kmicikewycz and Heinrichs, 2014). One could hypothesize that the higher fiber content of the TMR in addition to the late stage of lactation of the cows in the present study resulted in the modest reduction in rumen pH when compared with the aforementioned experiments. For instance, similarly to this study, Krause and Oetzel (2005) had the same stages within periods, and used supplemental wheat and barley (1:1) pellets for the grain challenge. However, our base TMR was 35 to 37% NDF, whereas the one in Krause and Oetzel (2005) was 28 to 29% NDF. In the present study, cows averaged 245 DIM at the beginning of the experiment, different than 154 DIM for Krause and Oetzel (2005) or 104 DIM for Kmicikewycz and Heinrichs (2014). The late stage of lactation of the cows in this study and therefore lower intakes represented by this stage may have minimized treatment differences.

Feed restriction may have destabilized the ruminal microbial flora due to starvation of some bacteria (Van Kessel and Russell, 1997). Such a phenomenon of ruminal pH depression had previously been observed in experimental grain challenge to induce SARA in Holstein lactating cows (Dohme et al., 2008).

Figure 1. Ruminal, urine, and fecal pH least squares means for rumen-cannulated cows (CAN) that received the high treatment (HG) during the grain challenge (S3) and recovery stage (S4).

It has been postulated that DMI will be reduced by depressed rumen pH. Effects associated with this DMI depression have been summarized by Desnoyers et al. (2009) and include decreased frequency and amplitude of ruminal contractions inducing a relative increase in rumen load, increased rumen lactic acid and osmotic pressure, and inflammation of the ruminal epithelium, metabolic dehydration, decreased blood bicarbonate reserves, or alteration in the metabolic acid-base status. However, we only observed a modest difference in DMI among treatments, perhaps because rumen pH was never lower than 5.5 that was observed by Oetzel (2004). Therefore, the acidotic condition we have created may not have triggered any of the above physiological changes in either HOL or JER cows. This finding is also supported by health observations of cows during and after the induction.

Dry matter intake as a percentage of BW followed the same patterns as DMI. However, the interaction of stage by treatment was significant for HOL and a tendency overall (Table 3). Jersey cows had higher values for DMI as percentage of BW than HOL. Aikman et al. (2008) reported that the passage of feed through the gastrointestinal tract happened more quickly in Jerseys when compared with Holsteins, and the estimated particle breakdown and subsequent passage out of the rumen were faster in Jerseys. The authors also concluded that the difference in rate of passage was associated with increased eating and rumination time per kilogram of DM or NDF consumed by Jerseys.

Milk yield was modestly affected in the present study. Kmicikewycz and Heinrichs (2014) did not see any differences in milk production and milk components among treatments after a grain challenge and feeding cows with different sources of hay. The authors indicated that feeding supplemental long hay may be a suitable strategy to help maintain DMI during incidents of and recovery from periods of low ruminal pH. Milk fat percentage depression was not observed, in accordance with Krause and Oetzel (2005). Milk protein percentage, milk protein yield, milk lactose percentage, and milk lactose yield had similar response to restriction feeding as milk fat and milk fat production, respectively.

High concentrations of VFA in the rumen could potentiate metabolic acidosis where blood pH can be significantly decreased (Gianesella et al., 2010). Cows have a relatively small lung capacity and rely heavily on the kidneys to excrete excess hydrogen ions. Positive relationships have been shown between blood pH and urinary pH, and between ruminal pH and urinary pH, respectively (Roby et al., 1987; Füll, 1993). Cowles (2013) found a positive relationship between rumen and urine pH (mean ruminal pH = $3.25 + 0.377$ mean urinary pH; $r^2 = 0.15$, $P < 0.02$). Literature data are equivocal regarding the usefulness of the link between urine pH and rumen pH. Enemark (2008) reported that, in an unpublished study, a correlation existed between urine and rumen pH ($r = 0.28$, $n = 323$, $P < 0.01$). In contrast, Kricziokat et al. (2009) found a weaker

Figure 2. Rumen pH least squares means and associated standard errors for cows receiving control (CON), moderate (MG) or high (HG) treatments from -2 to 21 h relative to feeding on challenge day (S3). Treatment: $P < 0.01$; treatment $\times$ time: $P = 0.94$; linear treatment effect: $P < 0.01$; quadratic treatment effect: $P = 0.85$.

Figure 3. (A) Relationship between rumen pH and urine pH between -2 to 21 h after feeding. The connecting points in the graph indicate sequential data at 1-h intervals, between the start, grain challenge (star), and finish of the challenge period. Data set includes all treatments observations from rumen-cannulated cows (CAN). (B) Relationship between rumen pH and urine pH between -2 to 2 h after feeding. Urine pH = $6.2916 + 0.2949$ rumen pH; $R^2 = 0.77$, $P = 0.049$.

relationship between rumen and urinary pH ($r = 0.19$, $n = 348$, $P < 0.001$). However, the latter was based on rumen samples collected via stomach tube under field conditions.

Morgante et al. (2009) reported no urine pH depression during SARA between 4 to 6 h postfeeding. Urinary pH generally reflects the acid-base state of an animal; thus, monitoring urine pH can be an inexpensive and sensitive method to monitor the effect of diet on blood

pH (Goff and Horst, 1998). Decreased urine pH may be attributed to the declining in blood HCO_3^- and greater urine net acid excretion, implying that the acid load of lactating cows increased dramatically as fresh feed was ingested, and was excreted in urine. Moreover, blood pH is maintained in such a narrow range that the respiratory and excretory systems must respond quickly to any acid-base challenge (Hu et al., 2007).

We assume that blood and organs buffer systems are able to adapt to the change 2 h after feeding, so that the urine pH starts to increase; however, as a larger size meal was ingested again (when cows had access to feed after milking), the urine pH decreased again (Figure 1). These results show that the urine changed dramatically during the day. To better understand the physiological associations of rumen pH and urine pH, urine sampling should be obtained with more frequency in the first 2 h after feeding (Figure 3).

Treatments did not affect urine pH in Jersey cows, whereas the treatment effect was significant for HOL cows. However, we did not have Jersey cows' rumen pH data to develop a similar formula to describe relationship between urine and rumen pH. Escobosa et al. (1984) did not see urine pH differences for Jersey cows or Holstein cows that were fed high-chloride or high-sodium diets during the hot weather. The authors reported that Jerseys had higher respiratory rates and lower body temperatures than Holsteins. Unfortunately, the authors were not able to measure water intake for Holstein and Jerseys, but it seems that the breeds have different electrolytic adaptation strategies. In the present study, fecal pH did not change for JER when compared with HOL (Table 4).

Hindgut acidosis occurs when excessive carbohydrate fermentation in the large intestine leads to an accumulation of organic acids (Gressley et al., 2011). Fecal consistency changes in response to hindgut acidosis, including diarrhea, frothy feces, and presence of mucin casts, which can be used in the field as indicators of SARA (Plaizier et al., 2008). The pH of the feces is usually slightly acidic (Dirksen, 1986). Li et al. (2012) reported that daily average pH decreased in the feces of cows experiencing SARA induction. We observed different time points after the grain challenge, and found that the fecal pH pattern was similar to that of rumen pH but lagged in time. Even though not statistically tested, HOL cows had stronger responses to treatments than JER cows; this may be related to the amounts of fermentable carbohydrate passed to the lower gut in each breed. Additionally, JER cows had faster response due to treatments than HOL cows, this may have happened because the smaller breed has a smaller digestive tract than larger size breed. However, in relative terms both breeds should have had similar DMI/digestive

tract ratios [JER, DMI = 13.2 kg/d, gastrointestinal tract = 25.83 kg (Smith and Baldwin, 1974), ratio = 0.511; HOL, DMI = 18.2 kg/d, gastrointestinal tract = 33.79 kg (Smith and Baldwin, 1974), ratio = 0.540], and therefore the explanation for such response is not clear.

In the present experiment, we found that the time points of 15 and 23 h after feeding were associated with rumen pH change based on fecal pH for HOL cows, whereas the time points of 7, 15, and 23 h after feeding were associated with rumen pH change based on fecal pH, for JER cows. However, we did not monitor the fecal pH frequently. More research is needed to identify the best time point for practical use.

CONCLUSIONS

Linear and quadratic treatment responses to a grain challenge in Holstein and Jersey cows were found for rumen and urine pH. Grain challenge reduced urine pH in Holstein cows but not in Jersey cows. The DMI and health of Holstein cows were not affected when rumen pH was depressed to between 5.6 and 5.8 for less than 4.3 h. A potentially useful link between rumen pH and systemic (urine) pH within 2 h after feeding was quantified in Holstein cows. Fecal pH could be associated with rumen pH depression as early as 7 h for Jersey cows and it was associated with rumen pH depression 15 h after feeding for Holstein cows.

ACKNOWLEDGMENTS

This project was partially supported by the American Jersey Cattle Association Research Foundation (Reynoldsburg, OH). The authors are grateful for the University of Illinois researchers, veterinarians, and the Dairy Research Unit staff involved in collection of the data.

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