Effects of clay after a grain challenge on milk composition and on ruminal, blood, and fecal pH in Holstein cows

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ABSTRACT

Oral supplementation of clay has been reported to function as buffer in dairy cows. However, its effects on rumen, blood, and fecal pH have varied among studies. Our objective was to determine the effects of 3 concentrations of dietary clay supplementation after a grain challenge. Ten multiparous rumen-cannulated Holstein cows [body weight (mean $\pm$ standard deviation) = 648 $\pm$ 12 kg] with 142 $\pm$ 130 (60 to 502) days in milk were assigned to 1 of 5 treatments in a replicated 5 $\times$ 5 Latin square design balanced to measure carryover effects. Periods (21 d) were divided into an adaptation phase (d 1 to 18, with regular total mixed ration fed ad libitum) and a measurement phase (d 19 to 21). Feed was restricted on d 18 to 75% of the average of the total mixed ration fed from d 15 to 17 (dry matter basis), and on d 19 cows received a grain challenge. The challenge consisted of 20% finely ground wheat administered into the rumen via a rumen cannula, based on the average dry matter intake obtained on d 15 to 17. Treatments were POS (no clay plus a grain challenge), 3 different concentrations of clay (0.5, 1, or 2% of dietary dry matter intake), and control (C; no clay and no grain challenge). Statistical analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Contrasts 1 (POS vs. C) and 2 (POS vs. the average of 0.5, 1, or 2%) were compared, along with linear and quadratic treatment effects. Rumen, fecal, and blood pH, along with blood metabolites, were measured at 0, 4, 8, 12, 16, 20, 24, 36, and 48 h relative to the grain challenge. Cows fed POS had lower rumen pH [(mean $\pm$ standard error) 6.03 $\pm$ 0.06] than cows fed C (6.20 $\pm$ 0.06). Cow fed POS had lower fecal pH (6.14 $\pm$ 0.04) than cows fed C (6.38 $\pm$ 0.04). We observed a linear treatment effect for rumen pH and fecal pH. Fecal pH (6.22 $\pm$ 0.04) was higher for cows fed clay (contrast 2) than for cows fed POS (6.14 $\pm$ 0.04).

We also observed a treatment difference (contrast 2) for negative incremental area under the curve, pH below 5.6 $\times$ h/d, (0.5% clay = 7.93 $\pm$ 0.83, 1% clay = 8.56 $\pm$ 0.83, and 2% clay = 7.79 $\pm$ 0.83) compared with POS (11.0 $\pm$ 0.83). Cows fed clay tended to have higher milk yield (0.5% clay = 28.8 $\pm$ 3.4 kg, 1% clay = 30.2 $\pm$ 3.4 kg, and 2% clay = 29.1 $\pm$ 3.4 kg, contrast 2), and had higher 3.5% fat-corrected milk (0.5% clay = 29.9 $\pm$ 3.5 kg, 1% clay = 34.1 $\pm$ 3.5 kg, and 2% clay = 33.1 $\pm$ 3.4 kg), and higher energy-corrected milk (0.5% clay = 29.1 $\pm$ 3.3 kg, 1% clay = 32.8 $\pm$ 3.4 kg, and 2% clay = 31.6 $\pm$ 3.3 kg) than cows fed POS (27.7 $\pm$ 3.4 kg, 28.0 $\pm$ 3.4 kg, 27.7 $\pm$ 3.3 kg, respectively). In conclusion, cows fed clay had higher rumen pH, energy-corrected milk, fat-corrected milk, and a trend for milk yield than cows fed POS.

Key words: buffer, clay, rumen pH, grain challenge

INTRODUCTION

Dietary ingredients in dairy cow diets affect animal efficiency and health. To produce milk at maximum efficiency, concentrates are required as a feed choice, but a high inclusion of concentrate in the TMR has gained popularity (Eastridge, 2006). Increasing concentrate-to-forage ratios and more elaborate grain processing in lactating dairy cow diets have been associated with higher milk production (Khorasani and Kennelly, 2001; Yang et al., 2001). However, too much concentrate can challenge the cow's natural buffering capacity and leave the rumen susceptible to drastic drops in pH levels (Shaver et al., 2000). Knowing the diurnal rhythm of rumen pH is crucial to understanding when a cow confronts SARA (Enemark, 2008). The minimum rumen pH fluctuates from 5.4 to 6.6, making it difficult to distinguish what is truly SARA (Duffield et al., 2004; Krause and Oetzel, 2006). Gozho et al. (2005) have defined SARA as when rumen pH is between 5.2 and 5.6 for at least 3 h/d. Cows facing SARA may experience symptoms such as decreased DMI and milk production, altered milk composition, diarrhea, and laminitis (Duffield, et al., 2004; Gozho et al., 2005;

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Krause and Oetzel, 2006; Plaizier et al., 2008). Even though SARA is difficult to diagnose, it is estimated to be prevalent in 19 to 26% of early- and mid-lactation dairy cattle (Enemark, 2008; Plaizier et al., 2008). Changes in rumen pH bring about changes in microbial populations that may lead to a decrease in milk fat percentages (Krause and Oetzel, 2005; Weimer et al., 2010). Additionally, the feces of cows with SARA have a lower DM content, longer particles, whole grains, a sweet-sour smell, and appear to be brighter, yellowish, and liquid (Oetzel, 2000; Kleen et al., 2003; Li et al., 2012). It is thought that these alterations are caused by postruminal fermentation in the intestines due to a massive outflow of fermentable carbohydrates from the rumen (Oetzel, 2000; Plaizier et al., 2008). Mixing feed additives (buffers) into the diet of dairy cows has been proven to alleviate the adverse health effects of SARA (Ghorbani et al., 1989; Shaver et al., 2000; Enemark, 2008; Cruywagen et al., 2015). Buffers have been used primarily to maintain rumen environments with a pH >5.0 and <7.0 (Shaver et al., 2000).

Clays are the products of silicate rocks that have been subjected to weathering processes for thousands of years (Buckman and Brady, 1969). Many types of clays have been used in diets as buffers; “silicate” is a specific term to describe clay. The main classification is phyllosilicates, which consists of many subcategories: kaolinite, smectites, chlorites, and micas (Adamis and Williams, 2005). Each term describes the chemical composition: for example, kaolins are usually composed of quartz and mica and have been used to treat digestive problems, and bentonites have been used as enterosorbents (Trckova et al., 2004). Bentonites consist primarily of a mineral called montmorillonite, which has an articulated layer structure with a net negative charge, balanced by cations within the interlayer space. Montmorillonite has an attraction for mono- and divalent ions that bind proteins and nitrogenous compounds to bypass the rumen (Wester, 2002; Trckova, et al., 2004; Adamis and Williams, 2005). Adding bentonite to corn before ensiling at 0.5 and 1.0% of the wet weight significantly increased the pH of the corn silage (Everson et al., 1971).

Understanding how rumen, blood, and fecal pH are affected by clay after a grain challenge in Holstein cows and its effect on production parameters deserves attention. The objectives of this experiment were (1) to determine the effects of a commercially available clay product after a grain challenge on ruminal, blood, and fecal pH, as well as on milk composition, in mid-lactation Holstein cows; and (2) to determine the most appropriate clay concentration to be used in the diet of lactating dairy 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 was September to December 2014. Cows were housed in tie stalls with sand bedding and ad libitum water and feed access. Mineral salt blocks were available at all times in the alleys from the tie stalls to the milking parlor (Big 6 Mineral Salt; North American Salt Company, Overland Park, KS). The diet (TMR) was formulated according to NRC (2001) recommendations.

Experimental Design and Grain Challenge Procedure

A total of 10 multiparous rumen-cannulated Holstein cows [BW (mean $\pm$ SD) = 648 $\pm$ 12 kg] with 142 $\pm$ 130 (60–502) DIM were assigned to 1 of 5 treatments in a replicated 5 $\times$ 5 Latin square design balanced to measure carryover effects. Treatments were arranged so that carryover effects could be evaluated. Periods (21 d) were divided into an adaptation phase (d 1 to 18, with regular TMR fed ad libitum) and a measurement phase (d 19 to 21). Day 18 had restricted feeding (cows were offered 75% of the average of the TMR fed on d 15 to 17, DM basis), and on d 19 a grain challenge occurred. A schematic representation of the experimental phase is shown in Figure 1. The grain challenge was similar to the one used for SARA induction proposed by Kmicikewycz and Heinrichs (2014). A grain challenge consisted of finely ground wheat administered via rumen cannula at a level of 20% of the average DMI obtained on d 15 to 17. Treatments were no clay plus a grain challenge (**POS**), 3 different concentrations of clay (0.5, 1, or 2% of dietary DMI), and a no clay and no grain challenge (control, **C**).

The clay used in this experiment was analyzed by inductively coupled plasma mass spectrometry and had the following mineral content (percentage of DM): magnesium 7.2, silicon 6.3, aluminum >5, iron 6.9, potassium 0.5, and manganese <0.1. Ion chromatography was used to report the presence of other chemical functional groups. The clay's ionic composition (mg/kg) was sulfate 124, chloride 113, carbonate 641, nitrate 97, and phosphate 2 (Avomeen Analytical Services, Ann Arbor, MI).

All cows were fed the same TMR throughout the trial, and were fed once daily at 1400 h. The daily clay allocation was weighed every day to correlate to kilograms of TMR offered, and equal portions were offered

Table 1. Ingredient composition of the lactation diet fed to cows in positive control with no clay (POS), 0.5, 1, or 2% clay, or negative control (C) treatments throughout the experimental period

Ingredient	% of DM
Alfalfa hay	8.20
Corn silage	33.6
Alfalfa silage	7.20
Wet brewers grain	7.49
Dry ground corn grain	21.55
Soybean meal, 48%	3.25
Expeller soybean meal ¹	3.30
Soy hulls	10.42
Limestone	0.13
Dicalcium phosphate	0.35
Bypass fat ²	1.87
Biotin	0.34
Molasses, sugar beet	2.05
Salt (plain)	0.04
Mineral and vitamin mix ³	0.20

¹SoyPlus (West Central, Ralston, IA).
²Energy Booster 100 (Milk Specialties Global, Paris, IL).
³Formulated with 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg/kg of Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg of Se, 2,200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D₃, and 7,700 IU/kg of vitamin E.

at 0600 and 1400 h. Each portion was mixed with 0.5 kg of ground corn and top dressed on the TMR. Cows in the C and POS treatment groups were given a top dress consisting of 0.5 kg of ground corn only.

Data Collection and Sampling Procedures

Samples of feed ingredients and TMR (Tables 1 and 2) were obtained on the first day of each week and analyzed for DM (AOAC International, 1995a) by drying for 24 h in a forced-air oven at 110°C. Diet composition was adjusted weekly for changes in DM content. The TMR offered and refused from each cow was recorded to determine intake based on weekly DM analyses. Daily DMI was used to calculate daily clay allocation. Samples of TMR delivered to each cow, n = 10) were taken on d 15 of each period and stored at -20°C until analyzed. Composite samples (3 per period) were analyzed for contents of DM, CP, ADF, NDF, lignin, starch, fat, ash, 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-Listing-Alphabetical-July-2015.pdf>)). The 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 the first day of each week.

Cows were milked 3 times daily at 0400, 1200, and 2200 h. Milk weights were recorded at every milking, and samples were obtained from d 15 at each of the

Figure 1. Schematic design of each experimental period and its sampling time points (days, D, 15 to 21). Milk = composite milk sample, R = rumen sample, F = fecal sample, B = blood sample. Similar diets were fed the entire period before the grain challenge. Color version available online.

corresponding milkings. A preservative was added to each milk sample, and samples were refrigerated for 3 d (800 Broad Spectrum Microtabs II; D&F Control Systems Inc., San Ramon, CA). The preserved samples were composited in proportion to milk yield at each sampling and sent to a commercial laboratory (Dairy Lab Services, Dubuque, IA) to be analyzed for fat, protein, lactose, urea N, total solids, and SCC using mid-infrared procedures (AOAC International, 1995b).

Health evaluations were conducted daily during the last week of each period for the duration of the challenge. Visual assessments were conducted to monitor general appearance and fecal score. Rectal temperature was measured using a GLA M700 thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). Respiration rate was recorded by watching the cow breathe for 15 s, and heart rate was measured using a stethoscope for 15 s. General appearance was scored using a method similar to Krause et al. (2009): 4 = bright and alert; 3 = depressed; 2 = reluctant to rise; 1 = down cow, will not get up. Fecal scores were allocated on a scale of 1 to 4 according to Krause et al. (2009): 1 = runny: liquid consistency, splatters on impact, spreads readily; 2 = loose: may pile slightly and spreads and splatters moderately on impact and setting; 3 = soft: piles up but spreads slightly on impact and settling;

4 = dry: hard, dry appearance, original form not distorted on impact and settling. 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 ; and fecal score was considered abnormal if ≤ 2 (Ireland-Perry and Stallings, 1993; Krause and Oetzel, 2005).

Body weight was measured (CW-11 digital scale; Ohaus, Newark, NJ) and BCS was assigned in quarter-unit increments for each cow weekly (Ferguson et al., 1994). More than 1 person assigned a BCS independently at each time of scoring, and the average score was used for statistical analysis. Cows were fitted with an activity logger (Hobo Pendant G Acceleration Data Logger; Onset Computer Corp., Pocasset, MA) positioned laterally on the distal portion of the left hind leg. The activity logger was attached to the leg using vet wrap. Data points were set to record at 60 s intervals. Data collected were used to calculate total lying time, number of lying bouts (number times lying down/24 h), and duration of lying bouts (time from lying down to standing up), as well as standing time, standing bouts (number times stood/24 h), and duration of each standing bout (time from standing up to lying down).

Table 2. Mean chemical composition and associated standard deviations for diets fed to cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments throughout the experimental period

Item	Period ¹					SD ²
	1	2	3	4	5	
DM, %	48.4	44.5	47.3	46.4	43.1	2.62
CP, % of DM	15.8	14.5	15.1	14.8	14.6	0.80
ADF, % of DM	22.5	22.0	22.2	22.2	24.2	0.40
NDF, % of DM	32.4	32.7	32.6	34.1	31.6	0.60
Lignin, % of DM	4.0	3.3	4.1	3.4	3.9	1.20
Starch, % of DM	27.4	29.9	28.8	28.9	27.4	1.30
Crude fat, % of DM	5.5	4.9	5.2	4.9	4.4	0.49
Ash, % of DM	6.54	6.89	6.06	6.38	7.06	0.56
TDN, % of DM ³	72	73	73	73	71	2.12
NE _L , Mcal/kg of DM ³	1.71	1.71	1.74	1.74	1.66	0.06
Ca, % of DM	0.71	0.76	0.64	0.66	0.69	0.06
P, % of DM	0.40	0.30	0.31	0.31	0.35	0.02
Mg, % of DM	0.22	0.20	0.19	0.20	0.26	0.02
K, % of DM	1.29	1.16	1.07	1.02	1.41	0.15
Na, % of DM	0.23	0.26	0.23	0.21	0.22	0.07
S, % of DM	0.22	0.19	0.19	0.20	0.21	0.01
Fe, mg/kg	711	699	522	631	674	325
Zn, mg/kg	94	89	84	93	85	5.66
Cu, mg/kg	17	17	16	17	17	2.12
Mn, mg/kg	90	96	77	86	87	3.51
Mo, mg/kg	0.7	0.7	1.0	1.0	1.2	0.35

¹Period = 3 consecutive weeks. Cows were fed TMR ad libitum. Each cow was given a mixture of 500 g of ground corn and clay at 0, 0.5, 1, or 2% of dietary DM. The clay was split into 2 top dress amounts and given immediately after feeding and again after 14 h.

²Maximum within-period SD.

³NRC (2001).

Rumen fluid (500 mL) was extracted via rumen cannula by pumping a representative sample (ventral sac, cranial sac, and caudo-ventral blind sac) into a graduated cylinder using a siphon for immediate pH measurement with a portable pH meter (AP110, Fisher Scientific, Pittsburgh, PA). Samples were collected at 0, 4, 8, 12, 16, 20, 24, 36, and 48 h relative to the grain challenge (time point 0) on d 19 of each period. At the same time points, fecal samples (approximately 40 g wet weight) were collected from the rectum of each cow into plastic containers, and fecal pH was measured immediately after collection with the portable pH meter (AP110, Fisher Scientific). Blood samples were collected from the coccygeal vein or artery at 0, 16, 24, and 48 h relative to the grain challenge. Blood samples were collected into tubes containing heparin sulfate and placed on ice. Heparin sulfate tubes were taken to the University of Illinois clinical pathology laboratory within 10 min of collection for blood gas analysis to measure pH, partial pressure of CO₂ (pCO₂), partial pressure of O₂ (pO₂), base excess, HCO₃⁻, total CO₂ (tCO₂), O₂ saturation, and lactate (photometric analysis performed according to the laboratory procedures on the AU680 Chemistry Analyzer, Beckman Coulter, Brea, CA). Additional samples were collected into tubes (BD Vacutainer; BD and Co., Franklin Lakes, NJ) containing a clot activator for serum and dipotassium EDTA for plasma. Serum and plasma samples were obtained by centrifugation at 2,500 × *g* for 15 min and stored at -20°C until further analysis.

Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (v 9.3; SAS Institute Inc., Cary, NC) to account for carryover effect using 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 observation 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 carryover 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 using 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} was the observation for dependent variables; μ was the general mean; S_i was the fixed effect of the i th treatment sequence; $A_{(i)j}$ was the random effect of the j th cow in the i th sequence; $P_{(i)k}$ was the fixed effect of the k th period; T_l was the fixed effect of the l th treatment; D_m was the fixed effect of repeated measurement, which was used as time point in pH and day in DMI analysis; $T \times D_{lm}$ was the interaction of treatment and repeated measurement (the interaction was removed if $P > 0.3$); and $e_{(ijk)l}$ is the random error. The estimation method was restrictive maximum likelihood and the degrees of freedom method was Kenward-Rogers (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 was used in the model (Littell et al., 2002).

A log₁₀ transformation was used for milk SCC, lactate, pCO₂, and tCO₂ for better homogeneity of the distribution of residuals. Means shown in tables and graphs for these variables were back-transformed. Orthogonal contrasts were tested using the CONTRAST statement in SAS: contrast 1 was POS (0% clay) compared with C; contrast 2 was POS (0% clay) compared with the average of the 3 clay treatments (0.5, 1, or 2%) and linear and quadratic effects of treatments POS (0), 0.5, 1, or 2%. 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 5.6 as the baseline. Residual distribution was evaluated for normality and homoscedasticity. A multivariable logistic mixed model (FREQ procedure) was used for the dichotomized variables (fecal score, general appearance, temperature, respiration, and heart rate). The chi-squared value was computed. Statistical significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Diet Composition

The ingredient composition of the diets is detailed in Table 1. Analyzed nutrients from the experimental diet are shown in Table 2. The physical characteristics of the TMR was (mean ± SD) 4.8 ± 1% on upper, 38.6 ± 3% on middle, 40.7 ± 3% on lower sieves, and 15.8 ± 2% in the pan. Carryover effect was tested and was not present for any variable of interest ($P > 0.45$).

DMI and Lactation Performance

Performance data are shown in Table 3. Cows fed POS had lower ($P = 0.02$) DMI than cows fed C. We observed no difference in DMI among cows fed clay (0.5, 1, or 2%) compared with cows fed POS. Cows fed POS yielded less ($P = 0.009$) milk than cows fed C. Cows fed clay (0.5, 1, or 2%) tended to yield more milk ($P = 0.06$) and had higher 3.5% FCM ($P = 0.02$) and ECM ($P = 0.01$) than cows fed POS. Cows fed clay (0.5, 1, or 2%) produced more ($P = 0.05$) milk fat yield when compared with cows in POS. We observed a positive linear treatment ($P = 0.03$) effect on milk fat yield and an increase in fat (kg/d; $P = 0.05$) when cows were fed clay during a grain challenge. We observed a quadratic treatment effect ($P = 0.03$) for milk protein (kg/d), with cows fed C yielding more protein ($P < 0.01$). Cows fed clay (0.5, 1, or 2%) had a tendency ($P = 0.10$) for a linear treatment effect for 3.5% FCM/DMI, with clay 1% being numerically higher (for every 1 kg of DMI, 1.99 kg of 3.5% FCM milk was produced) than the others.

Blood Gas and Rumen, Blood, and Fecal pH

Table 4 and Figures 2, 3, and 4 show the results for blood gas and metabolites, and blood, rumen, and fecal pH. Rumen pH was different ($P = 0.003$) for cows fed C compared with cows fed POS. Cows fed clay (0.5, 1, or 2%) had higher ($P = 0.02$) rumen pH than cows fed POS. Time points differed ($P < 0.0001$) for rumen pH, fecal pH, base excess, and blood HCO_3 , as well as blood pH ($P = 0.001$). We observed a positive linear treatment effect ($P = 0.001$) on rumen pH and a tendency for a positive linear treatment effect ($P = 0.06$) for fecal pH. Nadir rumen pH tended to be lower ($P = 0.06$) for cows fed POS than cows fed C. Cows fed clay (0.5, 1, or 2%) had higher ($P = 0.03$) nadir rumen pH than cows fed POS. Cows fed POS spent more time ($P = 0.007$) with rumen pH below 5.6 (are under the curve) than cows fed C. Cows fed clay (0.5, 1, or 2%) spent less time ($P = 0.005$) with rumen pH below 5.6 than cows fed POS. We observed a negative linear treatment ($P = 0.03$) effect for the time cows spent with rumen pH below 5.6. Fecal pH was different ($P < 0.0001$) between

Table 3. Least squares means and associated SEM for DMI, BW, BCS, and milk parameters response to a grain challenge for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C)

Item	Treatment (Trt) ¹						P-value ²			
	POS	0.5%	1%	2%	C	SEM	Contrast		Trt	
							1	2	Linear	Quadratic
DMI, ³ kg/d	16.97	17.96	16.63	17.52	19.00	1.14	0.02	0.55	0.81	0.84
BW, kg	643.9	648.4	650.7	648.2	652.1	12.2	0.44	0.54	0.71	0.58
BCS	3.04	3.23	3.11	3.08	3.24	0.10	<0.01	0.10	0.79	0.09
Milk yield										
Milk yield kg/d	27.72	28.77	30.20	29.08	30.58	3.4	0.009	0.06	0.18	0.06
3.5% FCM	28.24	29.85	34.13	33.09	30.00	3.4	0.42	0.02	0.01	0.14
ECM	27.71	29.05	32.82	31.56	29.63	3.3	0.27	0.01	0.01	0.09
Milk composition										
Fat, %	3.86	4.03	4.20	4.35	3.63	0.34	0.56	0.33	0.22	0.79
Fat, kg/d	1.00	1.08	1.29	1.26	1.04	0.13	0.74	0.05	0.03	0.25
Protein, %	3.01	3.00	2.99	2.92	3.05	0.09	0.49	0.42	0.10	0.62
Protein, kg/d	0.83	0.85	0.89	0.84	0.91	0.10	<0.01	0.16	0.73	0.03
Lactose, %	4.66	4.55	4.67	4.47	4.46	0.11	0.04	0.24	0.09	0.44
Lactose, kg/d	1.30	1.32	1.43	1.31	1.36	0.16	0.27	0.26	0.84	0.03
MUN, mg/dL	12.43	11.52	12.18	11.07	11.69	0.68	0.43	0.27	0.21	0.88
SCC, log ₁₀	4.30	4.12	4.26	4.48	4.74	0.54	0.37	0.98	0.60	0.61
3.5% FCM/DMI, kg/kg	1.66	1.67	1.99	1.85	1.61	0.14	0.77	0.15	0.10	0.23
ECM/DMI, kg/kg	1.62	1.61	1.91	1.76	1.59	0.13	0.79	0.19	0.14	0.20
Milk/DMI, kg/kg	1.63	1.57	1.78	1.62	1.63	0.14	0.91	0.64	0.71	0.13

¹Dietary treatments were positive control [POS, without clay (0%) and with grain challenge], 0.5% clay (0.5% of the dietary DMI as clay in a top dress), 1% clay (1% of the dietary DMI as clay in a top dress), 2% clay (2% of the dietary DMI as clay in a top dress), and negative control (C, without clay and no grain challenge). Top dress vehicle was 500 g of ground corn. Grain challenge = 20% of the average of the DMI of the last 3 d before the challenge as finely ground wheat.

²Contrasts: 1 = POS (0%) compared with C; 2 = POS (0%) compared with the average of the 3 treatments (0.5, 1, or 2%). Linear and quadratic effects of treatments POS (0%), 0.5, 1, and 2%.

³TMR was restricted to 75% of average DMI on d 18 of each period. These DMI do not include feed received as part of a grain challenge.

Table 4. Least squares means and associated SEM for pH and blood metabolites response to a grain challenge for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments

Item	Treatment (Trt) ¹					SEM	Contrast		P-value ²		Time point (TP)	Trt × TP
	POS	0.5%	1%	2%	C		1	2	Trt			
									Linear	Quadratic		
Rumen pH	6.03	6.05	6.16	6.20	6.20	0.06	0.003	0.02	0.001	0.53	<0.0001	0.01
pH <5.6, ³ h	6.36	5.60	4.57	4.88	4.16	1.26	0.32	0.37	0.41	0.55	—	—
Nadir pH	4.94	5.25	5.06	5.12	5.19	0.07	0.06	0.03	0.42	0.20	—	—
AUC, pH × h/d	11.0	7.93	8.56	7.79	7.71	0.80	0.007	0.005	0.03	0.14	—	—
Fecal pH	6.14	6.22	6.18	6.25	6.38	0.04	<0.0001	0.05	0.06	0.72	<0.0001	0.10
Blood pH	7.38	7.38	7.39	7.39	7.37	0.01	0.52	0.54	0.32	0.88	0.001	0.57
Partial pressure of CO ₂ (pCO ₂), mmHg	50.5	50.6	49.0	48.8	51.9	1.3	0.42	0.47	0.25	0.83	0.12	0.72
Partial pressure of O ₂ (pO ₂), mmHg	53.6	52.1	64.4	61.9	49.1	7.48	0.63	0.37	0.20	0.77	0.11	0.28
Base excess, mmol/L	4.61	4.37	4.39	4.36	4.82	0.48	0.70	0.60	0.71	0.77	<0.0001	0.93
HCO ₃ , mmol/L	29.7	29.5	29.3	29.4	29.5	0.48	0.80	0.57	0.62	0.69	<0.0001	0.93
Total CO ₂ , mmol/L	31.2	33.5	30.8	30.9	31.6	1.17	0.79	0.71	0.45	0.61	0.2	0.50
O ₂ saturation %	65.4	67.5	69.2	72.1	64.7	3.32	0.86	0.24	0.11	0.89	0.37	0.35
Lactate, mmol/L	1.09	1.13	1.05	0.92	1.13	0.16	0.77	0.45	0.17	0.82	0.06	0.11
Corrected ⁴ pH	7.36	7.36	7.39	7.37	7.35	0.01	0.76	0.31	0.35	0.25	0.009	0.29
Corrected pCO ₂ , mmHg	54.0	53.8	52.1	52.2	55.1	1.37	0.54	0.36	0.23	0.66	0.078	0.69
Corrected pO ₂ , mmHg	58.6	56.7	71.9	67.4	53.8	7.95	0.62	0.35	0.20	0.67	0.11	0.26

¹Dietary treatments were positive control [POS, without clay (0%) and with grain challenge], 0.5% clay (0.5% of the dietary DMI as clay in a top dress), 1% clay (1% of the dietary DMI as clay in a top dress), 2% clay (2% of the dietary DMI as clay in a top dress), and negative control (C, without clay and no grain challenge). Top dress vehicle was 500 g of ground corn. Grain challenge = 20% of the average of the DMI of the last 3 d before the challenge as finely ground wheat.

²Contrasts: 1 = POS (0%) compared with C; 2 = POS (0%) compared with the average of the 3 treatments (0.5, 1, or 2%). Linear and quadratic effects of treatments POS (0%), 0.5, 1, and 2%.

³During the first 24 h; at 0, 4, 8, 12, 16, 20, 24, and 48 h relative to grain challenge.

⁴Negative incremental area under the curve. Baseline rumen pH = 5.6.

⁵Corrected for cow's rectal temperature at time of sampling according to Ashwood et al. (1983).

Figure 2. Least squares means $\pm$ SE for rumen pH response to a grain challenge (0 h) for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments from 0 to 48 h (time points) relative to a grain challenge. Treatment: $P = 0.003$; contrast 1: $P = 0.02$; contrast 2: $P = 0.001$; linear treatment effect: $P = 0.53$; time point: $P < 0.0001$; treatment $\times$ time point: $P = 0.01$.

cows fed C and cows fed POS. Cows fed clay (0.5, 1, or 2%) had higher ($P = 0.05$) fecal pH than cows fed POS.

Health and Activity

We observed no differences for rectal temperature, general appearance, or fecal score among treatments ($P > 0.10$). Heart rate tended ($P = 0.10$) to be higher for cows fed POS than for cows fed C. Respiratory rate [(mean $\pm$ SE) 37.0 ± 1.1 breaths/min] was higher ($P = 0.02$) for cows fed clay (contrast 2) than cows fed POS (34.1 ± 1.7). Table 5 is an overview of the activity log of all cows throughout the trial. Cows fed clay tended to have less lying time ($P = 0.09$), more standing bouts ($P = 0.09$), and higher total standing time ($P = 0.09$) than cows fed POS. We observed a tendency for a quadratic treatment effect for standing bouts ($P = 0.08$) and standing time ($P = 0.07$).

DISCUSSION

The aims of this study were to determine the effects of a clay product after a grain challenge on ruminal, blood, and fecal pH, and on milk composition, in mid-

lactation Holstein cows; and to determine the most appropriate clay concentration to be used in the diet of dairy cows. We postulated that clay administration in different concentrations would alleviate the effects of a grain challenge and might affect the rumen or intestinal environment or modulate the acid-base metabolism, thereby affecting DMI, nutrient supply, metabolic responses, and performance in Holstein cows.

Cows in the present study encountered SARA when receiving a grain challenge (Gozho et al., 2005). Cows fed C had less area under the curve below rumen pH 5.6. These results were expected, because cows fed POS took longer to adjust their rumen environment to the normal pH range compared with cows fed C (Figure 2). Others have also been successful in reducing rumen pH after a grain challenge (Krause and Oetzel, 2005; Kmicikewycz and Heinrichs, 2014). Stage of lactation (DIM) seems not to have interfered with the effectiveness of the grain challenge. In the present study, cows were in a later stage of lactation than previous experiments. The association of SARA 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

Figure 3. Least squares means $\pm$ SE for fecal pH response to a grain challenge (0 h) for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments from 0 to 48 h (time points) relative to a grain challenge. Treatment: $P < 0.01$; contrast 1: $P = 0.05$; contrast 2: $P = 0.06$; linear treatment effect: $P = 0.72$; time point: $P < 0.0001$; treatment $\times$ time point: $P = 0.10$.

been extensively reported (Kleen et al., 2003; Al-Zahal et al., 2007; Kmicikewycz and Heinrichs, 2014). In the present study, cows that received the grain challenge had lower DMI than cows that did not receive the grain challenge (contrast 1; Table 3).

In the meta-analysis by Hu and Murphy (2005), rumen pH increased when buffered diets were used compared with unbuffered diets. The higher contents of magnesium and aluminum silicate may have contributed to the buffering capacity of the clay in the present study. Clays have been shown to work as alkalinizers and have great capacity for H^+ exchange at different pH ranges (Yong et al., 1990). The authors reported that illite clay (a type of clay with high concentrations of magnesium and aluminum silicate) had the best buffer capacity in the pH range from 4.5 to 6, similar to the rumen pH range. Additionally, MgO when used as a buffer, may increase ruminal outflow, increasing the acetate:propionate ratio and improving milk fat tests (Davis, 1979).

Earlier reports from Rindsig et al. (1969) concluded that cows fed clay at 5% (dietary DMI) had increased acetate and decreased propionate in the rumen, leading

to significant increases in milk fat percentage. In our study, a positive linear effect of treatment on rumen pH indicated that clay at 2% was most efficient in buffering rumen pH and reducing the time spent below rumen pH 5.6 after a grain challenge. Greater concentrations of clay may have allowed for greater buffering capacity.

Clay's mode of action is commonly associated with its ion-exchanging capacity (Yong et al., 1990). For instance, clay materials are often used as backfill or buffer materials for radioactive waste disposal sites because of their ion-exchange properties, low permeability, and easy workability (Kumar and Jain, 2013). Hu and Murphy (2005) reported in a meta-analysis that buffers used in diets decreased molar proportions of propionate, which in turn increased the acetate:propionate ratio. Cruywagen et al. (2015) used buffered diets and reported a positive influence on milk fat as acetate was increased in the rumen.

Because clay buffers cause a shift in microbial population to favor acetate in the rumen, leading to an increase in milk fat, it can be hypothesized that the clay product used in the present study worked the same way (Rindsig et al., 1969; Ghorbani et al., 1989;

Table 5. Least squares means and associated SEM for lying and standing behavior for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments

Item	Treatment (Trt) ¹						P-value ²			
	POS	0.5%	1%	2%	C	SEM	Contrasts		Trt	
							1	2	Linear	Quadratic
Lying bouts, no./24 h	24.2	21.0	23.4	23.62	24.06	5.37	0.96	0.49	0.88	0.49
Lying duration, min	50.6	55.7	51.6	49.84	52.18	5.59	0.76	0.69	0.65	0.48
Lying time, min	821.5	750.3	785.5	763.5	787.2	37.8	0.35	0.09	0.29	0.29
Standing bouts, no./24 h	9.3	10.3	10.29	9.92	10.07	0.64	0.21	0.09	0.56	0.08
Standing duration, min	73.9	73.4	72.3	73.18	70.42	7.26	0.29	0.80	0.85	0.76
Standing time, min	657.6	725.4	700.4	681.7	663.8	39.5	0.84	0.09	0.83	0.07

¹Dietary treatments were positive control [POS, without clay (0%) and with grain challenge], 0.5% clay (0.5% of the dietary DMI as clay in a top dress), 1% clay (1% of the dietary DMI as clay in a top dress), 2% clay (2% of the dietary DMI as clay in a top dress), and negative control (C, without clay and no grain challenge). Top dress vehicle was 500 g of ground corn. Grain challenge = 20% of the average of the DMI of the last 3 d before the challenge as finely ground wheat.

²Contrasts: 1 = POS (0%) compared with C; 2 = POS (0%) compared with the average of the 3 treatments (0.5, 1, or 2%). Linear and quadratic effects of treatments POS (0%), 0.5, 1, and 2%.

Cruywagen et al., 2015). Although VFA were not the main objective of the study, milk fat was significantly increased for cows that received clay during a grain challenge. In addition to increases in milk fat, others have found increases in milk protein yield when cows were fed buffered diets. Cows from 246 to 308 DIM that

were fed either 1 kg or 1% sodium sesquicarbonate had increased milk protein yield, but not cows earlier in their lactation (Tucker et al., 1994; Clark et al., 2009).

Hu and Murphy (2005) reported that, with corn silage-based diets, cows ate more when fed sodium bicarbonate, and if corn silage was not the main forage,

Figure 4. Least squares means $\pm$ SE for blood pH response to a grain challenge (0 h) for cows in positive control with no clay (POS), 0.5, 1, or 2% clay, and negative control (C) treatments from 0 to 48 h (time points) relative to a grain challenge. Treatment: $P = 0.52$; contrast 1: $P = 0.54$; contrast 2: $P = 0.32$; linear treatment effect: $P = 0.88$; time point: $P = 0.001$; treatment $\times$ time point: $P = 0.57$.

sodium bicarbonate had no effect on DMI. Others have found similar results, in which buffers had little to no effect on milk production or DMI when the cow's diet was not composed primarily of corn silage (Rearte et al., 1984; Kairenius et al., 2015). Ehrlich and Davison (1997) reported a decrease in DMI when cows were fed 4% sodium bentonite; however, these cows were fed bentonite mixed with sorghum grain not mixed in with the TMR, which would explain the decrease. The present study had no effect on DMI when fed clay, but cows fed clay did have increased milk yield compared with POS. Cruywagen et al. (2015) found an increase in milk yield for cows fed a mixture of 0.8% sodium bicarbonate and 0.35% limestone. Cows fed clay in the present study tended to increase milk yield and milk fat without changing DMI, ultimately leading to an increase in 3.5% FCM and ECM. These effects may have happened in response to the buffering effects discussed above.

Fecal pH continues to be under-studied by researchers. In 1980, fecal pH was reported to increase when cows were fed a combination of 1.5% sodium bicarbonate and 0.8% magnesium oxide (Erdman et al., 1980). Results from the present study showed a similar increase in fecal pH. Enemark (2008) reported that fecal pH is not normally related to ruminal pH unless starch can bypass the rumen and result in hindgut fermentation. If enough carbohydrates were to bypass the rumen and reach the large intestine, cows would experience hindgut acidosis, in which the fecal consistency could change to frothy with mucin casts (Gressley et al., 2011). We found no significant differences in fecal consistency (score) in the present study; however, Li et al. (2012) reported that fecal pH decreased when cows experienced SARA.

In the present study, fecal pH was significantly higher for cows fed clay during a grain challenge. The addition of clay might have caused changes in postruminal fermentation because of reduced outflow of fermentable carbohydrates from the rumen, or binding of fluid in the intestinal lumen with high osmolality (Oetzel, 2000). On the other hand, Luan et al. (2016) conducted a similar study but found no differences in fecal pH. This could be explained by the modest reduction in rumen pH in that experiment. Fecal pH and rumen pH patterns in the present study were similar but showed a lag time (Figures 2 and 3). This delay may have been related to the amount of carbohydrates passing the rumen, even though fecal appearances had no physical characteristics known for hindgut acidosis (Plaizier et al., 2008).

Blood gas measurements showed that cow physiology can maintain blood pH and its components at constant levels (Dominiczak and Szczepanska-Konkel, 2014). In the present study, cows fed clay had blood pH values

closer to the reported normal range of blood pH, at 7.4 (Bigner et al., 1997). Although we observed no significant differences among treatments, each treatment was different at different time points. From Figure 4, the cows fed POS reached the lowest blood pH (7.33) of all treatment groups 12 h following grain challenge. The diet containing 1% clay reached a blood pH of 7.36 at the same time point and regained normal values by 24 h. Bigner et al. (1997) conducted a study in which 500 g of sodium bicarbonate and 343 g of sodium propionate oral treatments raised blood pH. An association between blood pH and HCO_3 has been noted, but in the present study, we found no link between blood pH and clay administration. We observed a tendency for a linear increase in O_2 saturation percentage (Table 4) as clay was included in the diet. According to Karnland et al. (2006), a fraction of extractable iron oxides derived from some clay structures, upwards of 3.5%, were extracted from montmorillonite samples found in Germany, Czech Republic, and India. Therefore, one could hypothesize that increased O_2 saturation could be derived from the increased clay inclusion in the diet. However, the simple fact that cows had a higher respiration rate when fed clay through the challenge phase could have explained the linear effect on blood O_2 saturation.

Cows that received clay tended to spend less time lying down and more total time standing, as well as having an increased number of standing bouts. DeVries et al. (2009) correlated standing time and lying activity with eating behavior. As the risk of acidosis becomes more prominent, the frequency of meals decreases and the duration of mealtime increases. In the present study, we observed no differences in DMI, but we did not measure mealtime.

CONCLUSIONS

Feeding clay helped alleviate the effect of a grain challenge on the rumen environment and ultimately affected the performance of Holstein cows. Cows fed 0.5, 1, or 2% clay tended to yield more milk and did yield more 3.5% FCM and ECM than cows fed POS. A positive linear treatment effect on rumen pH indicated that clay at 2% was most efficient in buffering rumen pH and reducing the time spent below rumen pH 5.6 after a grain challenge. Production and physiological parameters suggest that clay may be an alternative buffer in diets for dairy cows.

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