



Physiological effects of starter-induced ruminal acidosis in calves before, during, and after weaning

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ABSTRACT

The objectives were to nutritionally induce or blunt ruminal acidosis in young calves and to compare indicators of rumen and systemic health. Ten bull calves ($n = 5/\text{diet}$) were ruminally cannulated at 3 wk of age and received milk replacer and 1 of 2 calf starter diets that were designed to cause (AC; pelleted, 42.7% starch, 15.1% neutral detergent fiber, 57.8% nonfiber carbohydrates) or blunt (BL; texturized, 35.3% starch, 25.3% neutral detergent fiber, 48.1% nonfiber carbohydrates) ruminal acidosis. Mean birth weight was 38.7 ± 1.3 kg. Body weight and calf starter intake were measured weekly. Rumen contents were sampled at $-8, -4, 0, 2, 4, 8, 12,$ and 24 h relative to starter feeding during wk 6, 8, 10, 12, 14, and 16 of age. Blood was collected from the jugular vein during the same weeks for complete blood cell count, blood pH, and partial pressures of oxygen and carbon dioxide. Rate of starter consumption was assessed during wk 16. Marker systems were used to estimate liquid passage and volatile fatty acid absorption rates. Calves were slaughtered at 17 wk, and rumen tissue was collected and assessed for papillae length, width, and degree of tissue degradation. Mean ruminal pH \pm standard error was 5.37 ± 0.24 and 5.63 ± 0.24 for AC and BL calves, respectively. Lowest pH values were observed the week after weaning. Total ruminal volatile fatty acid concentrations were 131.5 and 124.8 ± 2.4 mM in AC and BL calves, respectively, and increased with age and time after feeding. Dry matter intake was lower in AC calves at wk 4 and remained lower through wk 16. Rate of starter consumption was also lower in AC calves at wk 16. Body weight also was also lower for AC calves from wk 5 through 16. Blood hemoglobin and hematocrit were lower in AC calves, but

other blood characteristics were not different. Rumen volume increased with age and tended to be greater in BL calves. Passage rate and papillae length and width were not different between diets, but AC calves experienced a greater degree of tissue degradation. Ruminal acidosis symptoms in calves appear similar to those in adult cattle, and the etiology of the disease seems to follow similar mechanisms. It is clear from this study that symptoms can be moderated by diet, but further research is needed to determine whether symptoms can be nutritionally prevented or whether calves that experience ruminal acidosis are more susceptible to the disease as adults.

Key words: calf, acidosis, pH

INTRODUCTION

The first investigations into the mechanisms of rumen development were reported in the 1950s as reviewed by Kertz et al. (2017). Feeding hay and grain both resulted in tissue development, whereas tissue development did not occur when calves were fed only milk (Warner et al., 1956). McCarthy and Kesler (1956) observed increasing VFA concentrations in the rumen and blood in with increasing hay and grain intake. Later studies confirmed that rumen papillae and mucosal development were greater in calves fed milk with grain compared with calves fed milk alone (Tamate et al., 1962). Greater tissue development increases the VFA absorption capacity, allowing calves to be weaned from expensive liquid feeds onto cheaper solid feeds at an earlier age (Sutton et al., 1963). This economic advantage led to the current practice of feeding highly fermentable grains to young calves.

In adult cattle, highly fermentable diets can cause ruminal acidosis, a metabolic disease that occurs when the concentration of VFA increases, thereby decreasing ruminal pH to such a degree that ruminal and systemic functions are affected (Krause and Oetzel, 2005). Cooper and Klopfenstein (1996) used 5.6 and 5.2 as pH thresholds to define subacute and acute acidosis,

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respectively. As ruminal pH declines below 5.6, ruminal bacteria are altered (Russell and Hino, 1985; Nocek, 1997), parakeratosis may develop on the surface of rumen papillae (Steele et al., 2011), tight junctions loosen (Meissner et al., 2017), and free LPS concentration increases and the immune responses are stimulated (Stefanska et al., 2018). In dairy cattle, these physiological effects result in reduced DMI as well as decreased milk, fat, and protein production (Bhattacharya and Warner, 1967; Krause and Oetzel, 2005).

High concentrations of VFA and reductions in ruminal pH have been observed in young calves, especially at weaning (Laarman et al., 2012; Suarez-Mena et al., 2015, 2016). Diets offered to calves have ranged from completely pelleted to ground or whole grains (Beharka et al., 1998; Suarez-Mena et al., 2016). In each case, rumen pH fell below 5.6 for variable periods of time during the weaning transition (Beharka et al., 1998; Laarman et al., 2012; Suarez-Mena et al., 2015). Questions remain as to whether low ruminal pH in calves also causes similar ruminal dysfunction as observed in adult cattle. Supplementing a concentrate diet with forage can raise mean rumen pH in calves (Castells et al., 2013; Kim et al., 2016), but the effect is not consistent (Laarman and Oba, 2011; Suarez-Mena et al., 2016). The objectives were to nutritionally induce or blunt ruminal acidosis in young calves and to compare indicators of rumen and systemic health. It was expected that calves would experience ruminal acidosis as defined by rumen pH <5.6, and also would also exhibit signs of reduced ruminal and systemic health.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the University of Wisconsin–Madison Institutional Animal Care and Use Committee (A005848). Ten Holstein bull calves born at the Marshfield Agricultural Research Station (Marshfield, WI) between June 17 and July 5, 2017, were used for this experiment.

Calves and Diets

Each calf was removed from their dam, weighed, and received 3.79 L of colostrum within 3 h of birth. Additional feedings of colostrum were offered until 48 h of age. Blood samples were collected between 48 and 72 h to determine serum total protein using a handheld refractometer (Atago, Kowloon, Hong Kong). Calves were included in the trial when serum total protein >5.5 g/dL. After 48 h, 1.90 L (227 g of DM) of milk replacer (22% CP, 20% fat; Land O'Lakes Inc., Arden Hills, MN) was offered via nipple bottle at 0700 and

1900 h for 6 wk and then at 0700 h for 7 d. The same amount was offered each feeding until weaning. Calves were weaned at 8 wk of age. Calves were housed in individual calf hutches (4.8 m²/calf) from birth to 8 wk and then divided superhutches (5.0 m²/calf) through 16 wk. Rubber mats were placed underneath individual hutches and superhutches to prevent calves from ingesting bedding and to facilitate fecal collection. Total fecal collection was performed for 3 consecutive days during wk 5, 7, 9, 11, 13, and 15. Collected feces were composited by calf by week, and subsamples were analyzed for DM and starch content. Water was provided ad libitum for the duration of the study. Body weight was recorded weekly.

The diets offered were designed to cause (**AC**; pelleted, 42.7% starch, 15.1% NDF, 57.8% NFC) or blunt (**BL**; texturized, 35.3% starch, 25.3% NDF, 48.1% NFC) ruminal acidosis. Complete nutrient composition of each diet is given in a companion manuscript (Gelsinger et al., 2019). Each starter was fed as a complete diet. No supplemental roughage was provided to calves on either diet. Calf starters were randomly assigned and offered to calves at 1 wk of age (6.6 ± 3.4 d). A measured amount of starter was offered daily at 0800 h and refusals were determined daily. A calf's daily allotment of starter was increased when refusal <200 g was recorded on 2 consecutive days. Calves were allowed ad libitum access to their assigned starter for the duration of the trial up to a maximum of 4,500 g/d.

At approximately 3 wk of age, fistulas were created and soft rubber cannulas (28 mm i.d.) were inserted according to the method described by Kristensen et al. (2010) except that an initial incision was created in the skin and the rumen tissue was sewn fast to the skin and allowed to heal for 5 d before incising the rumen and inserting the cannula. Cannulas were replaced by larger soft rubber cannulas (51 mm i.d.; Bar-Diamond Inc., Parma, ID) between 7 and 9 wk of age as fistula size increased. Calves were slaughtered at 17 wk of age. Rumen tissues from the left and right sides of the cranial ventral sac were collected immediately afterward and fixed in 10% neutral buffered formalin to measure papillae structure according to Lesmeister et al. (2004).

Sample Collection

Rumen contents were sampled at −8, −4, 0, 2, 4, 8, 12 and 24 h relative to starter feeding during wk 6, 8, 10, 12, 14, and 16. A calibrated pH electrode was inserted into the rumen before each collection to measure rumen pH. Contents were collected by placing the end of a soft rubber hose (12.7 mm i.d.) inside the rumen through the cannula. The hose was fitted to an

armed Erlenmeyer flask and vacuum was created using a handheld pump. Rumen fluid was squeezed through 4 layers of cheesecloth and aliquots were collected into sterile centrifuge tubes and stored at -20°C for later analysis. Total volume collected at each sampling was 15 mL. During the trial, it was suspected that AC calves prolonged starter consumption throughout the day compared with BL calves, which consumed their full starter allotment rapidly. Therefore, the mass of starter remaining in each calf's bucket was measured and recorded at 2, 4, 8, and 12 h postfeeding during wk 16.

Liquid passage rate was estimated using the cobalt EDTA as a marker. An attempt was also made to estimate VFA absorption rate using valerate as a marker as described by Resende Júnior et al. (2006). Briefly, a marker solution was created by dissolving 30 g of cobalt EDTA (Udén et al., 1980) in 300 mL of 99% valeric acid (Alfa Aesar, Tewksbury, MA) and deionized water was added to a final volume of 2.6 L. Sodium hydroxide was used to adjust the pH to 6.5. Each calf received 60 mL of the final solution intraruminally immediately following sample collection at 0 h. Samples (5 mL) of rumen fluid were collected at subsequent time points and stored at -20°C to determine cobalt and valerate concentrations.

Whole blood samples were collected into EDTA-containing vacutainers via jugular venipuncture the day before rumen content collections. Samples were placed on ice and immediately transported to the Marshfield Veterinary Laboratory (Marshfield, WI) for complete blood cell count. Additional samples were collected into sodium heparin containing vacutainers during wk 8, 10, 12, and 14 and analyzed for blood pH and partial pressures of oxygen and carbon dioxide using an ABL80 FLEX Blood Gas Analyzer (Radiometer America, Brea, CA).

Sample Analyses

Cobalt. Cobalt concentration of rumen fluid was determined using an Optima 8000 ICP-OES (Perkin Elmer, American Fork, UT). A standard curve (0.0–46.49 mg/kg) was created using assurance grade cobalt (Spex CertiPrep, Houston, TX) diluted in 10% trace metal grade nitric acid (Thermo Fisher Scientific, Waltham, MA). Samples of rumen fluid were thawed and centrifuged at $2,000 \times g$ for 15 min at 25°C . The supernatants were diluted 1:1 with deionized water and refrigerated before aspiration into the ICP-OES. Concentrations were determined by comparing spectra from each sample to spectra from the standard solutions.

VFA. Volatile fatty acid concentrations were determined for samples collected in wk 6, 8, and 10 using HPLC. Rumen fluid samples were thawed, vortexed, and 1.5-mL subsamples were pipetted into duplicate tubes and centrifuged at $12,000 \times g$ for 5 min at 25°C . Following centrifugation, 600 μL of supernatant was transferred into clean microcentrifuge tubes to which 600 μL of calcium hydroxide suspension [52.9 g of $\text{Ca}(\text{OH})_2$, 250 mL of H_2O] and 300 μL of cupric sulfate solution (50.0 g of CuSO_4 , 500 mL of H_2O) were added. Crotonic acid (2.0 g) was added to the cupric sulfate solution as an internal standard. Tubes were vortexed and stored at $-20^{\circ}\text{C} \geq 4$ h. Thereafter, tubes were thawed, centrifuged at $12,000 \times g$ for 10 min at 25°C , and 1,000 μL of each supernatant was transferred into tubes containing 30 μL of concentrated sulfuric acid. Tubes were vortexed and stored at $-20^{\circ}\text{C} \geq 4$ h. Tubes were subjected to an additional freeze-thaw cycle before a final centrifugation at $12,000 \times g$ for 10 min at 25°C . Supernatants were transferred into vials for HPLC analysis (RID-10A, Shimadzu USA Manufacturing Inc., Canby, OR). Resulting chromatographs were manually reviewed and molar concentrations of each VFA were calculated based on internal and external standard values.

Rumen Papillae. Portions of fixed rumen tissue from the left and right sides of the cranial ventral sac were used for histological analysis. Tissues were mounted in paraffin wax, sliced, processed, and stained using hematoxylin and eosin. Four slides were created per calf (2 per area). Lesion scores were assigned to each slide based on the extent of keratinization, cell mineralization, bacterial colonization of the tissue, and infiltration of immune cells (0 = healthy tissue, 5 = severe tissue degradation and inflammation). Additional portions of tissue from each region were shipped to the Pennsylvania State University for length and width measurement (5 papillae/area) according to Lesmeister et al. (2004).

Statistical Analysis

Histograms were created for each dependent variable to confirm normality. A natural log-transformation was performed to correct normality for red blood cell and neutrophil counts, papillae length and width, and lactate concentration. Of the samples analyzed for lactate, only 99 samples contained measurable concentrations. Therefore, values were averaged by week and time to allow analysis of diet and time variables separately. Reported values are back transformed. Model fitness was confirmed by reviewing histograms of the residuals for normal distribution. Proc Mixed in SAS (version 9.3,

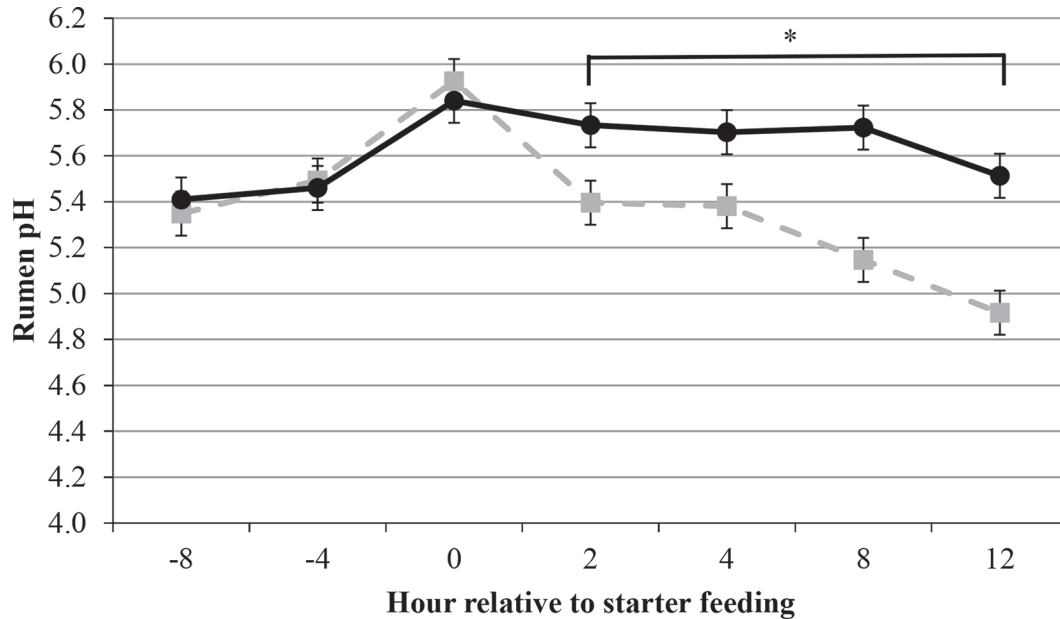


Figure 1. Least squares diet \times hour interaction means for rumen pH at different time points relative to feeding averaged over wk 6, 8, 10, 12, 14, and 16 of age. Calves were offered starter diets designed to cause (AC, gray dashed line, squares) or blunt (BL, black solid line, circles) ruminal acidosis from 1 to 16 wk of age. Hour $-8 = 0000$, $-4 = 0400$, $0 = 0800$, $2 = 1000$, $4 = 1200$, $8 = 1600$, and $12 = 2000$. *An asterisk indicates differences between diets ($P \leq 0.05$). Error bars indicate SEM.

SAS Institute Inc., Cary, NC) was used to analyze the following model for each dependent variable:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + e_{ijkl}$$

where μ = the overall mean of the population, α_i = the fixed effect of diet (AC, BL), β_j = the fixed effect of week of age (6, 8, 10, 12, 14, 16), γ_k = the fixed effect of sampling time (-8 , -4 , 0 , 2 , 4 , 8 , 12 , 24), and e_{ijkl} = the error term.

The effect of sampling time was removed for measurements collected once per week. Either week or sampling time within week was included as a repeated effect with calf or calf within diet as the subject. Covariance structures for each model were determined based on model convergence and Akaike's information criterion. The slice option was used to perform partial F -tests to determine differences between diets across time when significant interactions existed. Orthogonal contrasts were used to determine linear, quadratic, and cubic trends for the age effect. A chi-squared test was conducted using Proc Freq to create frequency tables for the number of times ruminal pH < 5.6 was observed in AC and BL calves. These calculated frequencies were compared with the null hypothesis of equal frequency across diets. Significance was declared when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$.

RESULTS

Mean ruminal pH was lower in AC compared with BL calves ($P < 0.01$). Despite a lower starch content and whole grain inclusion, BL calves experienced mean ruminal pH below 5.6 at 3 of 7 (wk 6, 10, 12, 16), 5 of 7 (wk 8), and 6 of 7 (wk 14) sampling times. A demonstrable change occurred in ruminal pH with time relative to starter feeding ($P < 0.01$; Figure 1). Ruminal pH peaked immediately before feeding (0 h). Nadirs were observed 12 or 16 h (-8 h) postfeeding for AC and BL calves, respectively. Mean ruminal pH decreased more rapidly postfeeding in AC calves and was lower compared with BL beginning 2 h postfeeding. Ruminal pH was lower in AC calves during the week of weaning (5.00 vs. 5.47; $P < 0.05$), but pH was not different between diets at other ages ($P \geq 0.12$). Ruminal values are given in Table 1.

Dry matter intake was lower in AC calves beginning at wk 4 and remained lower through wk 16 ($P \leq 0.04$; Figure 2). As a result, BW was also lower for AC calves from wk 5 through 16 ($P \leq 0.02$; Figure 3). Final carcass weights were 68.1 and 82.2 kg ($P < 0.01$) for AC and BL, respectively. Due to observations during sampling, rate of starter consumption was assessed during wk 16. The proportion of offered starter remaining in the buckets of calves fed each diet are shown in Figure 4. Calves fed the BL diet consumed 90% of their starter allotment within 2 h of feeding and the

remaining 10% by 8 h postfeeding. Alternatively, AC calves required the full 24-h period to consume their full allotment, consuming smaller proportions of their allotment throughout the day.

Propionate, isobutyrate, and total ruminal VFA concentrations were greatest in AC calves ($P \leq 0.04$; Supplemental Table S1, <https://doi.org/10.3168/jds.2019-17494>) and increased or tended to increase with age ($P \leq 0.10$). Acetate and butyrate concentrations were not different between diets ($P \geq 0.85$). Total ruminal VFA concentration increased with time after starter feeding ($P < 0.01$) driven by increases in propionate, acetate, isobutyrate, and butyrate ($P \leq 0.04$). An interaction was observed in total VFA concentration between sampling time and diet ($P = 0.04$; Figure 5). No 3-way interactions were observed ($P \geq 0.32$) for any VFA. Total VFA concentration peaked in AC calves at 2 and 12 h postfeeding. Total VFA concentration increased more slowly in BL calves with a smaller peak at 2 h and a larger peak 16 h (–8 h) postfeeding. Total VFA concentration was greater in AC calves at 2 and 12 h postfeeding ($P \leq 0.05$), which coincides with lower ruminal pH in AC calves. Lactate was detectable in 99 of 210 samples. Ruminal lactate concentration spiked in calves on both diets at 2 h postfeeding ($P = 0.04$). This likely explains the drop in ruminal pH observed in all calves at this time point. An attempt was made to measure valerate concentra-

tion as an indicator of VFA absorption rate (Resende Júnior et al., 2006); however, the dose of valerate was insufficient to raise the concentration above baseline levels and concentration diminished too quickly to estimate absorption coefficients. Therefore, we recommend a larger valerate dose and more frequent sampling schedule for future researchers attempting this method in calves.

Blood pH and partial pressure of carbon dioxide and oxygen were not different between diets; however, blood pH decreased linearly with age in all calves ($P < 0.01$; Table 2). Blood hemoglobin and hematocrit were lower in AC calves ($P < 0.01$; Table 2); however, red blood cell count was not different between diets ($P = 0.40$; Table 2), implying that red blood cell size was smaller in AC calves. Blood cell volume was not directly measured. Other blood cell counts were not different between diets ($P \geq 0.21$).

Rumen volume increased linearly with age ($P < 0.01$; Table 3) and tended to be greater in BL calves ($P = 0.08$; Table 3). A quadratic response of age was also observed in rumen volume ($P < 0.01$; Table 3). Rumen volume increased quickly for all calves in the 2 wk following weaning, then plateaued in BL and decreased in AC calves. Passage rate did not differ between diets ($P = 0.22$; Table 3) and demonstrated a quadratic response to age ($P < 0.01$; Table 3). Papillae length and width were not different ($P \geq 0.38$; Table 3), but a

Table 1. Diurnal ruminal pH values in calves at different weeks of age fed calf starter diets designed to cause (AC) or blunt (BL) ruminal acidosis

Age ¹ and diet ²	Time relative to starter feeding ³ (h)							SE	P-value					
	–8	–4	0	2	4	8	12		Diet (D)	Age (A)	D × A	Hour (H)	D × H	A × H
6														
AC	5.25	4.68	5.68	5.68	5.73	5.10 ^b	4.90 ^b	0.24	<0.01	0.12	0.69	<0.01	<0.01	<0.01
BL	5.20	5.10	5.50	6.00	6.12	5.88 ^a	5.62 ^a							
8														
AC	5.26	5.40	5.54	5.18	4.96	4.38 ^b	4.32 ^b							
BL	5.36	5.46	5.80	5.78	5.56	5.20 ^a	5.10 ^a							
10														
AC	5.42	5.70	5.66	5.42	5.58	5.44	5.10							
BL	5.40	5.54	6.16	5.98	5.84	5.62	5.48							
12														
AC	5.60	5.78	5.76	5.38	5.44	5.62	5.30							
BL	5.40	5.58	5.92	5.78	5.84	5.84	5.48							
14														
AC	5.60	5.94	6.46 ^a	5.34	5.24	5.28	4.74							
BL	5.54	5.40	5.76 ^b	5.54	5.42	5.58	5.38							
16														
AC	4.96	5.46	6.46	5.38	5.34	5.06 ^b	5.14 ^b							
BL	5.56	5.68	5.90	5.32	5.44	6.22 ^a	6.02 ^a							

^{a,b}Means within a week of age at a given time with different superscripts are different ($P \leq 0.05$). The 3-way interaction was not significant.

¹Week of age (6 to 16).

²AC = pelleted starter (42.7% starch, 15.1% NDF); BL = texturized starter (35.3% starch, 25.3% NDF).

³–8 = 0000 h, –4 = 0400 h, 0 = 0800 h, 2 = 1000 h, 4 = 1200 h, 8 = 1600 h, 12 = 2000 h.

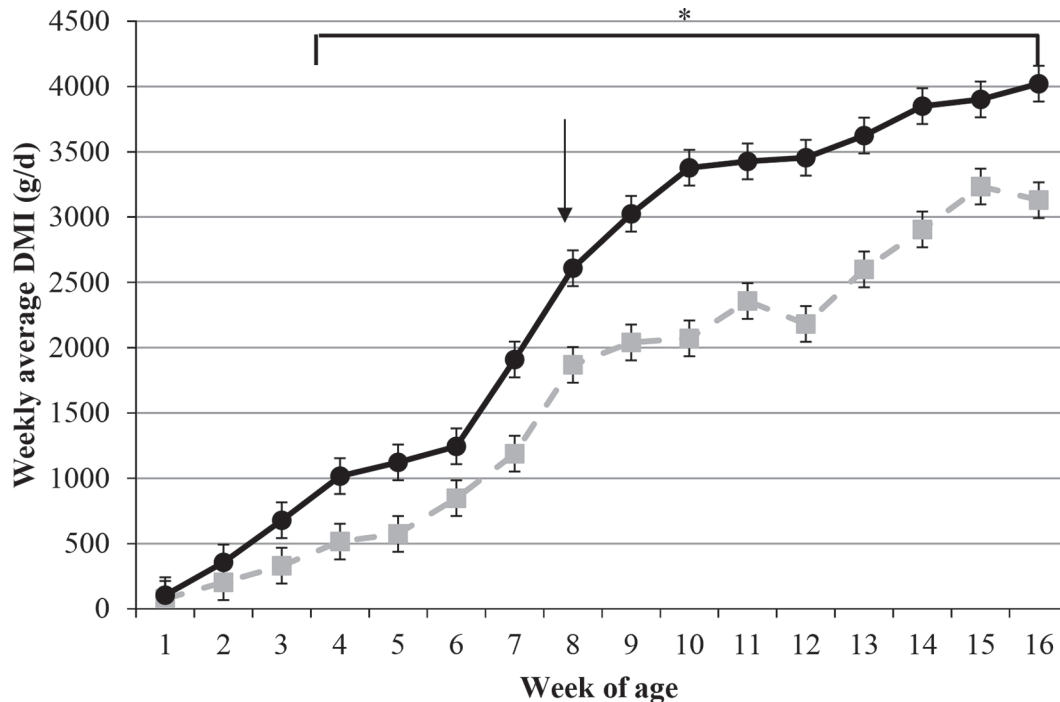


Figure 2. Mean weekly starter intake of calves from 1 to 16 wk of age offered diets designed to cause (AC, gray dashed line, squares) or blunt (BL, black solid line, circles) ruminal acidosis. *An asterisk indicates differences between diets ($P \leq 0.05$). Arrow indicates weaning. Orthogonal contrasts indicated linear ($P < 0.01$), quadratic ($P < 0.01$), and cubic ($P < 0.01$) effects of age. Error bars indicate SEM.

greater degree of tissue degradation was observed in AC calves ($P < 0.01$; Table 3).

DISCUSSION

The objective of this study was to use diet to induce or blunt ruminal acidosis in young calves and compare indicators of rumen and systemic health between the 2 diets. Keunen et al. (2002) demonstrated that ruminal acidosis could be induced by feeding ground wheat and barley and could be prevented by feeding less processed feeds. Similarly, in the current study, calves consuming a high-starch, pelleted diet (AC) exhibited lower mean ruminal pH compared with calves consuming a diet with whole grains and more fiber (BL). Although ruminal acidosis was not completely prevented in BL calves, ruminal pH < 5.6 occurred less frequently (90 vs. 133 observations, $P < 0.01$) and was reduced to a lesser extent compared with AC calves. Overall mean ruminal pH was 5.37 in AC and 5.63 in BL calves ($P < 0.01$). Therefore, AC calves are inferred to have experienced more severe acidosis than BL calves. Additionally, calves fed the AC diet exhibited other signs of ruminal acidosis associated with adverse rumen health in adult cattle including reduced DMI, reduced performance as measured by BW gain, altered feeding rate, and rumen epithelial lesions (Bhattacharya and Warner, 1967;

Krause and Oetzel, 2005; Steele et al., 2011). Ruminal lesions were also observed in BL calves, but to a lesser extent. Inclusion of physically effective fiber can reduce the prevalence of parakeratosis (Suarez et al., 2007). It may be that inclusion of whole grains in the BL diet provided sufficient physically effective fiber to reduce but not eliminate parakeratosis. Supplemental roughage was not supplied to calves in this study. Physiological mechanisms leading to ruminal parakeratosis are not completely defined. Parakeratosis is observed in calves with reduced rumen pH (Gilliland et al., 1962); however, raising pH by sodium hydrogen phosphate infusion did not prevent parakeratosis (Hinders et al., 1961). In culture, epithelial cell damage occurred when media were acidified using VFA but did not occur when media were acidified using gluconate (Meissner et al., 2017). Others also report a relationship between exposure to high levels of fat and increased intestinal permeability (Amado et al., 2019). It is clear that the degree of lesions calves experience can be manipulated via nutrition; however, it is not possible from this study to determine whether ruminal lesions may be prevented during the weaning process by feeding a different diet. It may be that all calves experience some degree of ruminal lesions as a result of the sharp increase in DMI and therefore, increasing ruminal VFA concentrations, at weaning. Diets in this study contained relatively high

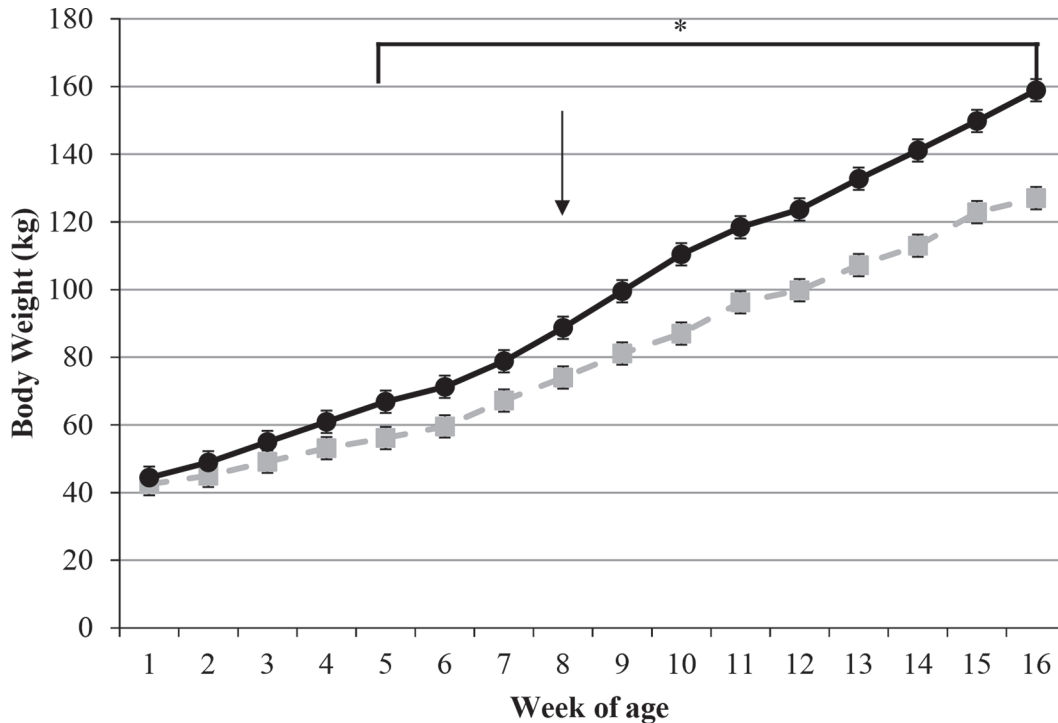


Figure 3. Weekly BW measurements of calves 1 to 16 wk of age that were offered starter diets designed to cause (AC, gray dashed line, squares) or blunt (BL, black solid line, circles) ruminal acidosis. *An asterisk indicates differences between diets ($P \leq 0.05$). Arrow indicates weaning. Orthogonal contrasts indicated linear ($P < 0.01$), quadratic ($P < 0.01$), and cubic ($P = 0.02$) effects of age. Error bars indicate SEM.

NFC concentration. Less fermentable diets or provision of supplemental roughage may prevent development of ruminal lesions (Suarez et al., 2007). Further research is needed using a variety of diets to determine whether lesions can be fully prevented.

Ruminal pH values reported here are similar to those reported by others at or around weaning (Beharka et

al., 1998; Suarez-Mena et al., 2016). Beharka et al. (1998) reported nadir ruminal pH of 5.3 and 5.5 for ground and unground diets, respectively. Suarez-Mena et al. (2016) also reported ruminal pH values in this range for calves offered starters containing 37 to 39% starch and 5% straw of various particle sizes. Suarez-Mena et al. (2016) and Beharka et al. (1998) also observed declining ruminal pH in calves approaching weaning similar to the current study. Anderson et al. (1987) demonstrated that this decline in ruminal pH is related to solid feed intake and occurs independent of age at weaning.

Vazquez-Anon et al. (1993) estimated rumen volume to be 5.8, 7.3, and 13.2 L in calves 2, 4, and 8 wk after weaning. Fluid and solid passage rates ranged from 11 to 13% and 2 to 6%, respectively, and did not differ by week. Estimated rumen liquid volume and fluid passage rate for AC and BL calves were greater than these values, likely because DMI was greater in the current study. Despite weekly increases in rumen volume and passage rate, VFA concentration increased sufficiently from wk 6 to 10 ($P < 0.01$) to reduce ruminal pH.

Total ruminal VFA concentration is highly variable in young calves. Values as low as 13 mM (Anderson et al., 1987) are observed in early weeks of life and increase with age and increasing DMI. Suarez-Mena

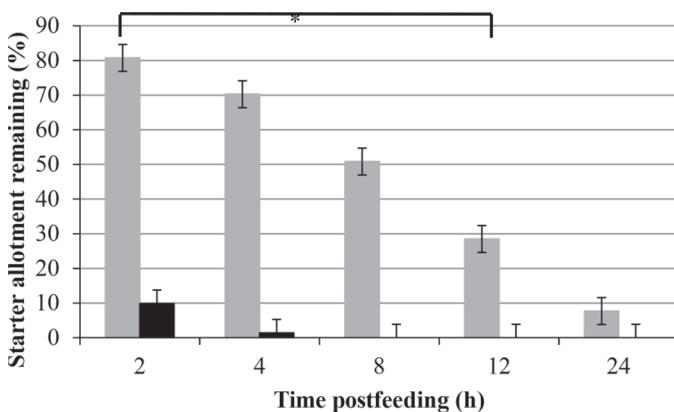


Figure 4. Percent of starter remaining at 2, 4, 8, 12, and 24 h post-feeding at 16 wk of age in calves offered diets designed to induce (AC, gray bars) or blunt (BL, black bars) ruminal acidosis. *An asterisk indicates differences between diets ($P \leq 0.05$). Error bars indicate SEM.

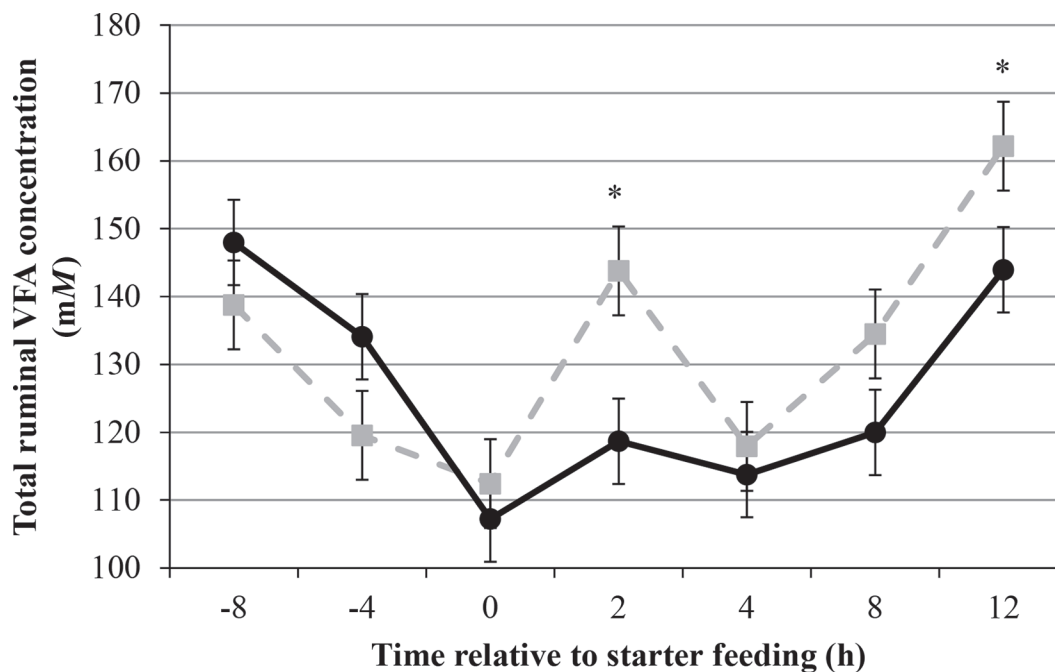


Figure 5. Least squares diet \times time means depicting changes in total rumen VFA concentrations over a 24-h period as measured during wk 6, 8, and 10 in calves fed starter diets designed to cause (AC, gray dashed line, squares) or blunt (BL, black solid line, circles) ruminal acidosis. Hour $-8 = 0000$ h, $-4 = 0400$ h, $0 = 0800$ h, $2 = 1000$ h, $4 = 1200$ h, $8 = 1600$ h, and $12 = 2000$ h. *An asterisk indicates differences between diets ($P \leq 0.05$). Error bars indicate SEM.

Table 2. Blood pH and partial pressures of CO_2 and O_2 in calves fed starter diets designed to cause (AC) or blunt (BL) ruminal acidosis¹

Item and diet ²	Age (wk)						P-value					
	6	8	10	12	14	16	SE	Diet (D)	Week (W)	D \times W	Lin ³	Quad ³
pH ²												
AC	—	7.42	7.42	7.39	7.37	—	0.01	0.20	0.01	0.77	<0.01	0.80
BL	—	7.41	7.40	7.38	7.37	—						
pCO ₂ ⁴ (mmHg)												
AC	—	51.5	50.6	50.3	53.4	—	1.7	0.16	0.67	0.85	0.42	0.36
BL	—	49.5	49.1	50.1	50.3	—						
pO ₂ ⁴ (mmHg)												
AC	—	44.2	54.0	51.4	45.2	—	3.4	0.48	0.10	0.68	0.87	0.01
BL	—	49.4	51.0	54.0	47.0	—						
Hemoglobin (g/dL)												
AC	9.8	10.1	10.4	10.3	10.7	10.8	0.33	<0.01	0.15	0.95	<0.01	0.90
BL	10.7	10.8	10.7	11.0	11.3	11.2						
Hematocrit (%)												
AC	31.6	32.5	33.2	31.7	33.0	34.2	1.0	<0.01	0.57	0.90	0.85	0.40
BL	34.9	34.6	34.3	34.2	35.0	35.4						
RBC (cells $\times 10^6/\mu\text{L}$)												
AC	8.44	8.89	8.98	8.53	8.90	9.09	1.0	0.40	0.64	0.87	0.60	0.64
BL	8.74	8.84	8.55	8.38	8.68	8.75						

¹Blood samples were collected via jugular venipuncture 1 \times /wk at 1000 h.

²AC = pelleted starter (42.7% starch, 15.1% NDF); BL = texturized starter (35.3% starch, 25.3% NDF).

³Lin = linear contrast for the effect of week of age. Quad = quadratic contrast for the effect of age. Cubic contrasts were not significant for any variables.

⁴Values were determined using an ABL80 FLEX Blood Gas Analyzer (Radiometer America, Brea, CA).

Table 3. Rumen volume, passage rate, and structural characteristics in calves fed starter diets designed to cause (AC) or blunt (BL) ruminal acidosis

Item and diet ¹	Age (wk)							SE	<i>P</i> -value ²			
	6	8	10	12	14	16	17 ³		Diet	Lin	Quad	Cub
Rumen liquid volume (L)												
AC	4.3	7.5	10.6	14.1	11.2	12.0	—	2.56	0.08	<0.01	<0.01	0.79
BL	5.8	7.3	13.9	14.9	15.5	17.0	—					
Liquid passage rate (%/h)												
AC	8.1	11.9	16.0	10.5	13.1	9.8	—	4.20	0.22	0.80	<0.01	0.57
BL	9.0	18.0	15.3	17.7	16.3	9.3	—					
Papillae length (mm)												
AC	—	—	—	—	—	—	4.2	0.21	0.58	—	—	—
BL	—	—	—	—	—	—	3.9					
Papillae width (mm)												
AC	—	—	—	—	—	—	1.4	0.06	0.38	—	—	—
BL	—	—	—	—	—	—	1.3					
Lesion score ⁴												
AC	—	—	—	—	—	—	3.7	0.15	<0.01	—	—	—
BL	—	—	—	—	—	—	2.3					

¹AC = pelleted starter (42.7% starch, 15.1% NDF); BL = texturized starter (35.3% starch, 25.3% NDF).

²Lin = linear contrast for the effect of age; Quad = quadratic contrast for effect of age; Cub = cubic contrast for effect of age.

³All calves were slaughtered at 17 wk of age.

⁴Scores were based on extent of keratinization, cell mineralization, bacterial colonization of the tissue and infiltration of immune cells; 0 = healthy tissue, 5 = severe tissue degradation and inflammation.

et al. (2016) reported values as high as 160 mM in calves immediately before weaning. Postweaning values range from 91 to 177 mM and are generally greater than preweaning measurements because of increased DMI during and after weaning (Anderson et al., 1987; Laarman et al., 2012). Studies comparing VFA concentration between calves fed ground or whole grain diets demonstrate 10 to 20 mM greater concentration at different ages in calves fed ground diets, but these differences are not always significant (Beharka et al., 1998; Suarez-Mena et al., 2015). The current study aligns with these studies demonstrating greater VFA concentration in AC calves ($P = 0.04$).

Despite reduced ruminal pH, neither group of calves demonstrated signs of systemic acidosis. Blood pH decreased linearly ($P < 0.01$) with age but remained within the range of values reported by others in healthy calves around weaning (Xin et al., 1991). Additionally, partial pressure of CO₂ and O₂ were not different between diets and did not change with age ($P \geq 0.10$). Hemoglobin and hematocrit were consistently lower in AC calves at all ages ($P < 0.01$), but red blood cell count was not different between diets ($P = 0.40$). These results imply that AC calves experienced greater cell turnover resulting in a larger proportion of smaller red blood cells compared with BL calves. Reference ranges for hemoglobin and red blood cell count in cattle are 8 to 15 g/dL and 5 to 10 cells $\times 10^6/\mu\text{L}$ (Fielder, 2019); thus, all calves were within normal ranges at all time points. Others have reported hemoglobin and hemato-

crit values ranging from 10.4 to 11.5 g/dL and 30.0 to 34.1%, respectively (Brooks and Hughes, 1932; Marchesini et al., 2013; Daneshvar et al., 2015) in 8-wk-old calves and nonpregnant heifers. Marchesini et al. (2013) observed reduced hematocrit and hemoglobin in nonpregnant heifers when challenged with a diet containing 33.4% starch compared with 42.8% starch. This is in contrast to the current study. Rumen tissue measurements were not reported in that study.

CONCLUSIONS

Starter intake, BW gain, feeding rate, blood parameters, and rumen structural parameters were monitored as indicators of calf health and differences were detected between diets and ages in each parameter. Calves fed the AC diet consumed less starter at a slower rate, grew at a slower rate, and exhibited a greater degree of rumen tissue lesions compared with BL calves. Based on these observations, we conclude that calves experience ruminal acidosis symptoms similar to adult cattle and the etiology of the disease seems to follow similar mechanisms. It is clear from this study that symptoms can be moderated by feeding diets containing whole grains, less starch, and more NDF. Further research is needed to determine whether these symptoms can be prevented using nutritional strategies and whether calves that experience ruminal acidosis during weaning are more susceptible to the disease as adults.

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