ABSTRACT

This study evaluated pre- to postweaning ruminal structural development, fermentation characteristics, and acute-phase protein levels in calves with a high milk replacer (MR) feeding rate prior to weaning. Six ruminally cannulated Holstein bull calves were fed MR (150 g/L) at 15% of body weight (BW) in 2 equal volumes daily. Volumes were adjusted weekly based on BW. Calves were weaned using a 1-step weaning method, with MR decreased by 50% at the end of wk 5 and full weaning at the end of wk 6. Calf starter, chopped straw, and water were offered ad libitum. Intake was recorded daily, and BW was recorded weekly. From wk 5 to 12, ruminal pH was continuously measured using a ruminal pH bolus. Ruminal fluid was collected weekly from wk 5 to 12 for measurement of short-chain fatty acid concentrations and quantification of total bacteria and protozoa. Rumen papillae were obtained at wk 5, 6, 7, 8, and 12 for histological analysis. Serum amyloid A and lipopolysaccharide-binding protein were measured weekly. Data were analyzed using GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC), with week as a fixed effect and calf as a random effect. During the weaning step-down, starter intake was 3-fold higher and continued to increase until wk 12. Body weight increased from birth to wk 12; however, BW did not change during wk 6, 7, and 8, possibly due to low metabolizable energy intake caused by the weaning strategy. Preweaning ruminal pH was below 5.8 for approximately 936.3 ± 125.99 min/d, implying ruminal acidosis. Furthermore, ruminal pH below 5.8 reached a peak at wk 8 with 1,203.9 ± 227.65 min/d below pH 5.8 and slowly decreased to 388.1 ± 189.82 min/d below pH 5.8 at wk 12. Papillae surface area, length, and width increased during wk 12 compared with wk 5. Corneum thickness increased by week, whereas spinosum/basale thickness only increased during wk 8 compared with wk 5. The acute-phase protein concentration was highest at wk 1 and then decreased and remained constant until wk 12. In conclusion, even before step-down weaning, calves experienced ruminal acidosis despite low starter intake. Further, the observed prolonged ruminal pH depression suggests that dietary rumen adaptation after weaning can take several weeks in calves with a high MR feeding rate preweaning. The prolonged depressed ruminal pH did not affect acute-phase proteins and this finding, along with the other results, suggests that rumen epithelium barrier integrity is not compromised during weaning.

Key words: calf, pH, rumen development, acute-phase proteins

INTRODUCTION

During the preweaning period, the calf’s gastrointestinal tract functions similarly to that of a monogastric animal, whereby milk/milk replacer (MR) is digested and absorbed in the abomasum and small intestine to supply adequate nutrients (carbohydrate and protein) for maintenance and growth (Baldwin et al., 2004; Drackley, 2008; Benschop and Cant, 2009). When calves start to consume solid feed after weaning, the rumen is the major site of fermentation, providing short-chain fatty acids (SCFA) that account for up to 80% of the ruminants’ energy (Bergman, 1990). Thus, the calf gastrointestinal tract experiences dramatic morphological and metabolic adaptations during weaning, through the transition from milk to solid feed (Baldwin et al., 2004), to support the energy requirements of the calf.

When calf starter (CS) intake increases during and after weaning, it can lead to an accumulation of SCFA in the rumen (Aschenbach et al., 2011), especially when rumen development has been delayed preweaning. Such accumulations of SCFA can lead to decreased ruminal pH, which is unfavorable because sustained low rumen pH (5.5) can cause decreased OM fermentation (Cerrato-Sánchez et al., 2007). Low pH can also influence the cell layers within the stratum corneum (Baldwin...
and Jesse, 1991), one of the 4 cell layers of the rumen, and the tight-cell junction protein complexes within the granulosum (Kiddle et al., 1951). It has been commonly reported that an accumulation or extensive sloughing of the corneum occurs when ruminants are fed a high-grain diet (Steele et al., 2011, 2015). The granulosum is thought to control the permeability of the epithelium (Gaebel et al., 1989; Baldwin, 1998), and low ruminal pH may lead to compromised ruminal health (Kleen et al., 2004). Moreover, low ruminal pH can trigger a systemic inflammatory response due to the translocation of LPS into the peripheral circulation in mature animals (Khafipour et al., 2009a). Acute-phase proteins such as haptoglobin (Hp), serum amyloid A (SAA), and LPS-binding protein (LBP) are commonly used as indicators of inflammation from subacute ruminal acidosis, signaling that either gastrointestinal tract mucosa damage has occurred or immunogenic molecules such as LPS have entered the circulatory system (Khafipour et al., 2009b). When animals experience inflammation or have an activated immune system, the body reprioritizes nutrient partitioning away from growth or production, which therefore makes the animal less efficient (Kvidera et al., 2017). Despite the knowledge of ruminal pH during weaning (Laarman and Oba, 2011; Laarman et al., 2012; Kim et al., 2016a), studies monitoring pH adaptations after weaning in calves are limited. In addition, no studies have investigated the effects of ruminal pH during weaning on indicators of inflammation (i.e., acute-phase proteins) in calves.

We hypothesized that in calves (1) a delay in rumen structural development occurs up to 2 wk after weaning; (2) ruminal pH is consequently depressed up to 2 wk after weaning; and (3) an acute inflammatory response occurs during weaning and up to 2 wk after weaning due to the depressed ruminal pH, causing barrier dysfunction. The objective of this study was to characterize preweaning to postweaning ruminal structural development, fermentation characteristics, and acute-phase proteins.

**MATERIALS AND METHODS**

**Animals and Housing**

In this study, calves were cared for and handled in accordance with the Canadian Council on Animal Care (CCAC, 2009) regulations and the institutional animal care and use committee (University of Alberta, AB; AUP00002010). A total of 6 Holstein bull calves (45 ± 1.5 kg birth weight; mean ± SE) were obtained from a commercial dairy farm (Millet, AB, Canada) within 1 wk after birth (van Niekerk et al., 2018). It was estimated that 4 animals per time point per treatment would be sufficient to detect differences in ruminal pH response variables based on a power calculation, with 80% power and α of 5%, based on estimates of variance drawn from previous literature (Wood et al., 2015; Eckert et al., 2015). The calves were housed in individual pens (3.05 × 3.66) with rubber mats (2.42 × 2.42 m) and deep bedding with wood shavings at the Metabolic Unit (University of Alberta, Edmonton, AB, Canada). At wk 2, calves were fitted with rubber ruminal cannulas (2.8-cm diameter; Lesmeister and Heinrichs, 2004). Before arrival, calves received 3 L of reconstituted powdered colostrum containing 200 g of IgG (HeadStart, Saskatchewan Colostrum Company, Saskatoon, SK, Canada) within 1 h of birth, followed by a second feeding (3 L with 200 g IgG) at 12 h after birth. Thereafter, calves were fed 3 L of MR (150 g/L, 26% CP, 20% fat, 38% lactose as fed; Grober Nutrition, Cambridge, ON, Canada) containing dried whey protein concentrate, dried skim milk powder, animal and vegetable fat, dried whey powder, Grober Rearing Premix, soy lecithin, calcium chloride, DL-methionine, calcium carbonate, L-lysine, yeast autolysate, viable microbial product (982931), PEG 400 mono- and di-oleates, and selenium yeast, twice daily (0500 and 1630 h) for the first week of life. From the second week onwards, MR solution (150 g/L) was fed according to 15% of BW per day in 2 equal volumes via nipple bucket twice daily (0700 and 1900 h). Milk volumes were adjusted according to BW weekly. Calves began the weaning step-down at wk 6, with the MR solution being restricted to 50% of the previous week’s allocation. Calves were completely weaned by wk 7. This feeding regimen was designed to elicit weaning stress. Upon arrival, calves were given ad libitum access to textured calf starter (Trouw Nutrition Canada Inc., Strathmore, AB, Canada; Table 1), chopped straw (Skyline Harvest, Blumenort, MB, Canada, 1-inch chop length), and water from the second week onward.

Table 1. Chemical composition of starter and hay fed to dairy calves

<table>
<thead>
<tr>
<th>Item</th>
<th>Starter</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % as fed</td>
<td>89.4</td>
<td>92.5</td>
</tr>
<tr>
<td>DM basis, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>23.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Fat</td>
<td>4.5</td>
<td>1.0</td>
</tr>
<tr>
<td>ADF</td>
<td>9.3</td>
<td>53.9</td>
</tr>
<tr>
<td>NDF</td>
<td>15.1</td>
<td>75.7</td>
</tr>
<tr>
<td>Starch</td>
<td>32.9</td>
<td>1.7</td>
</tr>
<tr>
<td>NFC</td>
<td>50.8</td>
<td>9.4</td>
</tr>
<tr>
<td>ME, δ Mcal/kg</td>
<td>2.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

δCalculated using NRC (2001).

Data and Sample Collection

**Intake and Growth Performance.** Individual MR, CS, straw, and water intakes were recorded daily. Samples of CS and straw were each collected weekly. The CS and straw were composited by type and stored until chemical analysis. Individual BW were measured at the same time weekly.

**Ruminal Fluid and pH.** Ruminal fluid was sampled through the cannula using a plastic tube and a syringe 3 h after the morning feeding from the end of wk 5 to 12. Ruminal contents were filtered through 4 layers of cheesecloth, and 5 mL of ruminal fluid was frozen and stored at −20°C for SCFA analysis. An additional 2 mL of rumen fluid was collected and stored at −20°C for total bacteria and protozoa density quantification. Ruminal pH was measured continuously every 5 min throughout the experiment from wk 5 to 12 using a T9 pH bolus (Dascor, Escondido, CA) according to Penner et al. (2006). The pH bolus was removed weekly to download data and for recalibration.

**Rumen Tissue.** Rumenal tissue biopsies were collected 3 h after the morning feeding at the end of wk 5, 6, 7, 8, and 12, as described by van Niekerk et al. (2018). Briefly, calves were not sedated but were restrained in a calf chute during sampling. For ruminal tissue sampling, an Allis clamp (15 cm, Jorvet, Loveland, CO) was used to retrieve the dorsal coronary pillar through the cannula opening. This exposed the caudodorsal blind sac for sampling, which was done using surgical scissors. To ensure that whole papillae were obtained, the cut was made below the base of the papillae. After the samples were obtained, the tissue was washed with sterile PBS (pH 7.4). For histological analysis, 2 ruminal tissue samples at each time point were submersed in 10% formalin solution and stored at −20°C for total bacteria and protozoa density quantification. Ruminal SCFA and pH, and Total Bacteria and Protozoa Density.

**Serum Acute-Phase Proteins.** Blood samples were collected weekly from wk 1 to 12 via jugular venipuncture 1 h after the morning feeding. Blood was collected into a silica-coated clot-activating vacutainer tube (Becton Dickinson, Franklin Lakes, NJ), inverted for 30 s, and stored until clotted (±30 min) at room temperature. It was then centrifuged at 1,500 × g for 30 s, and stored at −20°C for analysis of Hp, SAA, and LBP.

Sample Analyses

**Feed Composition.** At the end of the trial, CS and straw composited samples were sent for analysis to Central Testing Laboratory Ltd. (Winnipeg, MB, Canada). The CS and hay samples were analyzed for moisture (AOAC International, 2000; method 930.15; analyzed, AOAC method 922.02; near-infrared method), CP (AOAC International, 2000; method 990.03), crude fat (AOCS, 2005; standard procedure Am 5-04), ADF (AOAC International, 2000; ANKOM method 08-16-06), NDF (AOAC International, 2000; ANKOM method 08-16-06; Van Soest et al., 1991, using α-amylase), starch (enzymatic; UV method), and minerals (modification of AOAC methods 968.08, 935.13A, and 985.01; AOAC International, 2000).

**Ruminal SCFA and pH, and Total Bacteria and Protozoa Density.** The ruminal fluid was analyzed for SCFA and lactate by the Metabolomics Innovation Centre (University of Alberta, Edmonton, AB, Canada) using nuclear magnetic resonance (NMR) spectrum analysis (Saleem et al., 2013). Briefly, 250 µL of thawed rumen fluid was mixed with 46.5 µL of a standard buffer solution (54% D2O; 46% 1.75 mM KH2PO4, pH 7.0 vol/vol containing 5.84 mM 2,2-dimethyl-2-silcepentane-5-sulfonate). Then, 250 µL was transferred to an NMR tube (3-mm SampleJet, Bruker Corp., Billerica, MA), and the 1H-NMR spectra were collected by an Avance III spectrometer (700 MHz, Bruker) equipped with a cryoprobe (5 mm, HCN Z-gradient pulsed-field gradient). The Chenomx NMR Suite Professional software package (ver 8.1, Chenomx Inc., Edmonton, AB, Canada) was used to quantify the 1H-NMR spectra.

The ruminal pH was averaged by day and then by week. The minimum, mean, and maximum pH were reported from the summarized data. Ruminal acidosis time span per day was determined from the pH data using 3 pH thresholds (5.8, 5.5, and 5.2; Penner et al., 2007) by calculating the duration span (min/d) for each day and averaged by week.

The repeated bead beating and column method was used to extract DNA from ruminal fluid (Yu and Morrison, 2004). First, the samples were treated with a cell lysing buffer that contained 4% SDS and were physically disrupted (zirconium beads, diameter 0.1 mm) using a Beads Beater (2,160 × g for 3 min using BioSpec Mini Beads Beater 16, BioSpec, Bartlesville, OK). Thereafter, DNA was isolated, precipitated, and purified (Qiamp fast DNA stool mini kit, Qiagen Inc., Germantown, MD), and the quantity and purity were evaluated (NanoDrop 1000, Nanodrop Technologies, Wilmington, DE). The DNA was stored at −20°C for further analysis.

Real-time PCR was used to estimate 16S rRNA and 18S rRNA gene copy numbers of total bacteria and protozoa, respectively, with SYBR Green chemistry (Fast SYBR Green Master Mix, Applied Biosystems, Foster City, CA) using a Viia 7 Real-Time PCR System (Thermo-Fisher Scientific, Waltham, MA). The spe-
cific primers used for total bacteria were ACTCCTAC-GGGAGGCAG (forward) and GACTACCAGGG-TATCTAATCC (reverse) with a melting temperature of 60°C and product size of 467 bp (Stevenson and Weimer, 2007). The specific primers used for protozoa were GCTTTCGWTGTTAGTGTTAT (forward) and C7TGCCCCTCYAATCGTWCT (reverse) with a melting temperature of 60°C and product size of 223 bp (Sylvester et al., 2004).

**Rumen Histology Measurements.** Rumen papillae were retrieved from the formalin (fixative) and submerged in PBS. Individual papillae or a row of papillae (depending on the size) were dissected. A stereo microscope (Olympus Stereo Microscope SZ2-ILST, Tokyo, Japan) and ScopePhoto were used to obtain images from 10 papillae per calf per sampling time point. Papillae were positioned to ensure that all of them fit into the image. After imaging, the papillae were placed back in the fixative. The lengths of the 10 papillae were measured from the highest point perpendicular to their base using ImageJ software (National Institutes of Health, Bethesda, MD). The papillae width was measured at the midpoint of the papillae. The 1-sided surface area of the papillae was measured by outlining the papillae from base to base, and the result was multiplied by 2 to obtain the 2-sided surface area.

Rumen papillae cell layer thickness was measured as described by van Niekerk et al. (2018). Ruminal tissue samples were dehydrated overnight and submerged in paraffin wax. Then, the tissue was sectioned in 4-μm-thick slices, and stained with hematoxylin and eosin (Steele et al., 2011). A minimum of 5 papillae per calf per sampling time point were imaged using a light microscope (Zeiss Axio Scope.A1, Oberkochen, Germany) and optronics digital camera (PictureFrame Ver, 2.3; Figure 1C) at ×10 and ×20 magnification. The images obtained from these samples were used to measure the epithelium, corneum, granulosum, spinosum, and basal layer thicknesses and to count the number of cells per layer using ImageJ software. The different layers were identified by their distinct characteristic features as described in Steele et al. (2011). Briefly, the layers were defined as follows. The stratum corneum was the outermost cell layer, with no nuclei and heavily stained pink, and the granulosum was the second outermost layer, with nuclei and with cells that lay perpendicular to the cells of the stratum spinosum. The stratum spinosum and basale were measured together because it was difficult to distinguish between them; these layers made up the cells between the stratum granulosum and the lamina propria. The thickness of the cell layers was measured 25% inwards from the base and from the tip of the papillae by drawing a perpendicular line (to the base and tip) at these 2 spots, and cell numbers were also counted at these 2 spots. This resulted in 20 measurements (4 measurements per papilla) per calf per time point. Whole papillae were scored for sloughing according to Steele et al. (2015). A score of 1 to 5 was assigned to each papilla: minor desquamation (score 1), minor desquamation of the corneum (score 3), and severe desquamation (score 5). All measurements were performed by 2 individuals for quality control, and the averages are reported.

Immunohistochemistry for detection of Ki67 antigen was performed on 4-μm tissue sections mounted on charged slides and using an automated staining instrument (Dako Autostainer, Agilent Technologies/Dako, Mississauga, ON, Canada). Following manual deparaffinization and rehydration, sections were treated with 3% hydrogen peroxide to quench endogenous peroxidase activity. Heat-induced epitope retrieval was accomplished using Tris/EDTA buffer (pH 9; Target Retrieval Solution, Agilent/Dako). Sections were incubated with mouse anti-human Ki67 monoclonal antibody (1:50 dilution, clone MIB-1, Agilent/Dako), followed by horseradish peroxidase-labeled goat anti-mouse/anti-rabbit polymer (EnVision Flex HRP, Agilent/Dako) detection and Nova Red chromogen (Vector Laboratories, Burlington, ON, Canada). Sections of normal bovine lymph node were used as positive tissue controls. For negative reagent controls, duplicate sections of each control and test tissue were subjected to the same immunohistochemistry procedure with substitution of antibody diluent alone for the primary antibody. Ten digital brightfield images per slide were acquired per calf per sampling time point using a Leica DMR microscope (Leica, Wetzlar, Germany) fitted with a QImaging MicroPublisher 5.0 Real-Time Viewing Camera (Teledyne Photometrics, Tucson, AZ) and using OpenLab Image Analysis software (Improvision, Guelph, ON, Canada) at ×40 magnification. The images obtained from these samples were used to measure an area that spanned the granulosum, spinosum, and basal layer and to count the number of proliferating cells in that area, using ImageJ software.

**Blood Acute-Phase Proteins.** Serum amyloid A and LBP concentrations were determined using ELISA kits (TP-802, Tri-Delta Diagnostics Inc., Morris Plains, NJ; HK503, HyCult Biotechnology, Uden, the Netherlands, respectively). The serum was diluted 500× for SAA and 100× for LBP measurements initially and the absorbance values were read at 450 nm (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA) for each duplicate sample. For SAA and LBP, the intra-assay coefficient of variation values were 5.0 and 5.2%, respectively. Serum Hp concentrations were measured
using the hemoglobin binding capacity with a Roche Cobas 6000 c501 automated biochemistry analyzer (Roche Canada, Laval, Quebec), that used a methemoglobin reagent made by the Animal Health Laboratory, University of Guelph as previously described (Skinner et al., 1991; Skinner and Roberts, 1994).

Statistical Analyses

Data were analyzed as repeated measures using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The model included week as a fixed effect and calf as a random effect. Data collected daily were summarized by week. Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Calf starter, straw, and water intake; total SCFA; lactate; minimum, mean, and maximum ruminal pH and pH thresholds; papillae surface area, length, and width; total bacteria and protozoa; SAA; and LBP were used to estimate linear relationships through Pearson correlation coefficient using the corrplot package (v0.84) in R (v3.6.1). The thresholds used for the Pearson correlation ($r$) were 0.8 to 1 as very strong, 0.6 to 0.79 as strong, 0.40 to 0.59 as moderate, 0.2 to 0.39 as weak, and 0 to 0.19 as very weak correlation for the absolute values of $r$. Significantly correlated data were analyzed using the regression procedure of SAS.

RESULTS

Intake and Growth Performance

Milk replacer intake increased over time due to the milk feeding protocol (Figure 1A). The maximum aver-
age reached was 1.44 ± 0.039 kg of DM/d during wk 5, just before step-down weaning. Water intake remained below 2 L/d during the preweaning period (Figure 1A). Thereafter, water intake increased, reaching up to 8.1 ± 0.51 L/d during wk 12. For the first 3 wk, calves consumed less than 0.1 kg/d of CS, and during wk 4 and 5, they consumed approximately 0.13 ± 0.024 kg/d (Figure 1B). During the step-down week, CS intake was 3-fold higher than the previous week. After weaning, CS intake steadily increased to 2.8 ± 0.07 kg/d during wk 12. Calves consumed minimal straw (1 to 10 g/d) during the preweaning period, and straw intake was approximately 0.03 to 0.04 kg/d thereafter (Figure 1B). During the preweaning and step-down periods, MR intake contributed to the majority of the ME intake (Figure 1C), while during the postweaning period, CS contributed to the majority of the ME intake. During wk 7, calves consumed less (P < 0.05) ME than wk 1 because it took calves 6 wk after weaning to consume the same amount of ME from CS that they consumed from MR just before weaning. Body weight increased (P < 0.001) over time, reaching up to 109.3 ± 3.0 kg at wk 12; however, BW were not significantly different between wk 5, 6, 7, and 8 (Figure 2A). During wk 6, calves only gained 0.22 ± 0.052 kg/d and calves lost 0.32 ± 0.0256 kg/d during wk 7 (Figure 2A). During wk 12, calves started to gain weight at a similar rate (approximately 1.2 kg/d) as in wk 4 and 5.

**Rumen Fermentation Characteristics and Total Bacteria and Protozoa**

Overall, total SCFA concentration was the greatest (P = 0.001) at wk 7, 10, 11, and 12 (Table 2). Butyrate, propionate, and isobutyrate were unaffected by week. Acetate proportion was higher (P < 0.001) in wk 5 than in wk 9, 10, 11 and 12, while valerate had lower (P = 0.003) proportions during wk 5 than in wk 9, 10, 11, and 12. Isovalerate was 2-fold lower (P = 0.013) at wk 7 compared with wk 9. No significant difference was found in lactate over time; however, lactate was numerically higher during wk 5. Maximum ruminal pH had a week-related effect (P = 0.003), reaching the highest pH during wk 12 and the lowest during wk 7 and 8 (Figure 3A). In regard to duration time per day below a threshold, pH was below 5.8 for 1,203.9 ± 227.65 min/d at wk 8, which was 3-fold higher than at wk 12 at 388.1 ± 189.82 min/d (Figure 3B).

No differences in total bacteria and protozoa density in ruminal fluid were detected from wk 5 to 12. Total bacteria density in rumen fluid was 11.1 ± 0.1, 11.2 ± 0.16, 10.9 ± 0.11, 10.9 ± 0.10, 10.8 ± 0.16, 10.9 ± 0.08, and 10.7 ± 0.23 log10/mL in wk 5 to 12, respectively. Ruminal fluid protozoa density was 3.5 ± 0.22, 3.4 ± 0.18, 3.6 ± 0.25, 3.6 ± 0.26, 3.4 ± 0.15, 3.6 ± 0.18, 3.6 ± 0.26, and 3.7 ± 0.10 log10/mL in wk 5 to 12, respectively.

**Rumen Histology Measurements**

Papillae surface area and length were the greatest (P < 0.001) during wk 12 (Table 3). Papillae width increased (P < 0.001) by week, with wk 5 being the smallest, wk 7 and 8 intermediate, and wk 12 the largest. Supplemental Figure S1 (https://doi.org/10.3136/jds.2020-19003) illustrates the papillae growth at different time points. Corneum thickness increased by week (P = 0.003; Table 3; Supplemental Figure S1). Spino-sum-basale thickness increased (P = 0.009) during wk 8 compared with wk 5. No difference was observed in papillae sloughing score or Ki67-positive cells per area over time.

---

**Figure 2.** Mean weekly (A) BW, and (B) ADG in dairy calves (n = 6) fed up to 1.4 kg/d of milk replacer that were weaned during wk 6. Error bars represent SEM and arrows indicate step-down weaning. Different letters represent a significant difference (P < 0.05) between weeks.
Table 2. Short-chain fatty acid (SCFA) characteristics and lactate throughout wk 5 to 12 in dairy calves (n = 6) fed up to 1.4 kg/d of milk replacer that were weaned during wk 6

<table>
<thead>
<tr>
<th>Item</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>SEM</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SCFA, mM</td>
<td>57.2a</td>
<td>92.0ab</td>
<td>113.6b</td>
<td>103.1b</td>
<td>105.7b</td>
<td>118.7b</td>
<td>123.4b</td>
<td>132.8b</td>
<td>12.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Acetate, mol/100 mol</td>
<td>58.8b</td>
<td>50.4ab</td>
<td>49.5b</td>
<td>49.8b</td>
<td>47.4b</td>
<td>47.0b</td>
<td>46.7b</td>
<td>43.2b</td>
<td>2.31</td>
<td>0.006</td>
</tr>
<tr>
<td>Butyrate, mol/100 mol</td>
<td>12.05</td>
<td>15.75</td>
<td>16.62</td>
<td>17.62</td>
<td>19.27</td>
<td>18.55</td>
<td>17.69</td>
<td>22.75</td>
<td>2.426</td>
<td>0.15</td>
</tr>
<tr>
<td>Propionate, mol/100 mol</td>
<td>26.44</td>
<td>30.89</td>
<td>30.56</td>
<td>28.76</td>
<td>29.38</td>
<td>30.51</td>
<td>29.43</td>
<td>29.43</td>
<td>1.802</td>
<td>0.69</td>
</tr>
<tr>
<td>Isobutyrate, mol/100 mol</td>
<td>0.73</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.81</td>
<td>0.67</td>
<td>0.57</td>
<td>0.55</td>
<td>0.094</td>
<td>0.39</td>
</tr>
<tr>
<td>Isovalerate, mol/100 mol</td>
<td>0.50</td>
<td>0.27</td>
<td>0.26</td>
<td>0.33</td>
<td>0.54</td>
<td>0.45</td>
<td>0.34</td>
<td>0.30</td>
<td>0.064</td>
<td>0.013</td>
</tr>
<tr>
<td>Valerate, mol/100 mol</td>
<td>1.44a</td>
<td>2.09ab</td>
<td>2.44ab</td>
<td>2.90ab</td>
<td>3.81b</td>
<td>3.80b</td>
<td>4.17b</td>
<td>3.80b</td>
<td>0.480</td>
<td>0.003</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>2.95a</td>
<td>0.64</td>
<td>0.43</td>
<td>0.30</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a,bDifferent letters represent a significant difference (P < 0.05) between weeks.

Values represent means.

**Serum Acute-Phase Proteins**

A week-related effect was observed for LBP (P = 0.004; Figure 4A), SAA (P = 0.002; Figure 4B), and Hp (P = 0.028; Figure 4C) concentrations. The concentration of LBP was the highest during wk 1 (4.58 ± 0.92 µg/mL), which was approximately 2-fold higher than during wk 4, 10, 11 and 12, which ranged from 1.79 to 2.04 µg/mL. The concentration of SAA was also the highest during wk 1 (394.84 ± 175.26 µg/mL) and lowest during wk 4, 6, 8, 9, 10, 11, and 12, ranging from 16.52 to 102.96 µg/mL. Similarly, the concentration of Hp was also the highest during wk 1 (1,013.3 ± 686.23 µg/mL) and lowest during wk 3 to 12, ranging from 2.11 to 9.70 µg/mL.

**Intake, Rumenal Structural Development, Fermentation Characteristics, and Acute-Phase Proteins**

The correlation analysis demonstrated that papillae length (r = 0.80, P < 0.001), width (r = 0.90, P < 0.001), and water intake (r = 0.92, P < 0.001) displayed strong positive correlations with CS intake, and that papillae width (r = 0.86, P < 0.001) had a strong correlation with water intake (Figure 5). Total SCFA (r = 0.62, P < 0.001) and papillae surface area (r = 0.75, P < 0.001) had strong positive correlations with CS intake and papillae surface area (r = 0.67, P < 0.001), and length (r = 0.70, P < 0.001) had a strong positive correlation with water intake. Moderately positive correlations were observed between mean ruminal pH and CS intake (r = 0.41, P = 0.03), between straw intake and papillae width (r = 0.41, P = 0.02), and between papillae width and total SCFA (r = 0.58, P < 0.001). Moderately positive correlations were found between water intake and total SCFA (r = 0.53, P = 0.003) and between total protozoa and pH threshold 5.5 (r = 0.41, P = 0.03). Calf starter intake (r = −0.44, P = 0.01) and water intake (r = −0.43, P = 0.02) had moderate negative correlations with SAA. Straw (r = −0.48, P = 0.01), water intake (r = −0.42, P = 0.0), and papillae width (r = −0.46, P = 0.01) had moderate negative correlations with total bacteria, and total protozoa and maximum pH (r = −0.40, P = 0.03) also had a moderate negative correlation. Regression relationships for dependent and independent variables that were significantly correlated are presented in Supplemental Table S1 (https://doi.org/10.3136/jds.2020-19003).

The weaning transition is a phase in a calf’s life during which the rumen experiences drastic morphological and metabolic adaptations (Baldwin et al., 2004), which the current study has also shown. Calves are fed CS in an effort to initiate rapid rumen development during this phase; however, in the current study, a 2-wk delay occurred afterweaning. When SCFA absorption is inadequate due to a delay in rumen development, accumulation of SCFA and reduced ruminal pH may occur, possibly leading to ruminal acidosis (Aschenbach et al., 2011), which was also observed in the current study. Despite the body of research on ruminal pH during weaning (Laarman and Oba, 2011; Laarman et al., 2011), which was also observed in the current study. Despite the body of research on ruminal pH during weaning (Laarman and Oba, 2011; Laarman et al., 2011; Kim et al., 2016a), studies monitoring ruminal pH adaptations in calves after weaning are limited. In addition, to our knowledge no studies have investigated the effect of ruminal pH during weaning on acute-phase proteins in calves. Thus, the objective of this study was to evaluate pre- to postweaning ruminal structural

**DISCUSSION**

development, fermentation characteristics, and acute-phase protein levels in calves fed a high MR feeding rate prior to weaning.

Calves consumed minimal CS during the first 5 wk of life, as a reduction of MR supply is necessary for CS intake to increase to greater amounts (3-fold increase

Figure 3. (A) Daily ruminal pH summarized by week: maximum (Max), mean, and minimum (Min) ruminal pH from dairy calves (n = 6) fed up to 1.4 kg/d of milk replacer that were weaned during wk 6. (B) The duration (min/d) the pH was below pH threshold 5.8, 5.5, and 5.2. Error bars represent SEM and arrows indicate step-down weaning. Different letters represent a significant difference (P < 0.05) between weeks.

Table 3. Morphometric ruminal papillae parameters and cell density of rumen epithelial strata throughout wk 5, 6, 7, 8, and 12 in dairy calves (n = 6) fed up to 1.4 kg/d of milk replacer that were weaned during wk 6

<table>
<thead>
<tr>
<th>Item</th>
<th>Week 5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>12</th>
<th>SEM</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilla surface area, mm²</td>
<td>527a</td>
<td>616a</td>
<td>1,056a</td>
<td>1,195a</td>
<td>7,682b</td>
<td>852.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Papilla length, µm</td>
<td>695a</td>
<td>723a</td>
<td>880a</td>
<td>948b</td>
<td>3,227b</td>
<td>247.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Papilla width, µm</td>
<td>418a</td>
<td>456ab</td>
<td>676b</td>
<td>687b</td>
<td>1,392c</td>
<td>59.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corneum thickness, µm</td>
<td>11.9a</td>
<td>12.0a</td>
<td>13.7ab</td>
<td>15.9ab</td>
<td>17.2b</td>
<td>1.29</td>
<td>0.003</td>
</tr>
<tr>
<td>No. of cells in corneum</td>
<td>2.05</td>
<td>2.13</td>
<td>2.35</td>
<td>2.67</td>
<td>2.64</td>
<td>0.187</td>
<td>0.036</td>
</tr>
<tr>
<td>Granulosum thickness, µm</td>
<td>17.4</td>
<td>18.5</td>
<td>20.6</td>
<td>19.5</td>
<td>19.3</td>
<td>1.01</td>
<td>0.17</td>
</tr>
<tr>
<td>No. of cells in granulosum</td>
<td>2.44</td>
<td>2.24</td>
<td>2.50</td>
<td>2.27</td>
<td>2.35</td>
<td>0.996</td>
<td>0.25</td>
</tr>
<tr>
<td>Spinosum/basale thickness, µm</td>
<td>46.8a</td>
<td>48.8ab</td>
<td>67.0b</td>
<td>70.1b</td>
<td>63.9b</td>
<td>4.73</td>
<td>0.009</td>
</tr>
<tr>
<td>No. of cells in spinosum/basale</td>
<td>4.71</td>
<td>4.65</td>
<td>6.24</td>
<td>6.42</td>
<td>5.72</td>
<td>0.455</td>
<td>0.049</td>
</tr>
<tr>
<td>Epithelium thickness, µm</td>
<td>63.4a</td>
<td>66.6ab</td>
<td>87.2bc</td>
<td>90.6c</td>
<td>85.2bc</td>
<td>5.28</td>
<td>0.006</td>
</tr>
<tr>
<td>Papillae sloughing score</td>
<td>1.6</td>
<td>2.2</td>
<td>2.4</td>
<td>2.6</td>
<td>2.5</td>
<td>0.27</td>
<td>0.14</td>
</tr>
<tr>
<td>Ki67-positive cells, n/mm²</td>
<td>739</td>
<td>719</td>
<td>661</td>
<td>715</td>
<td>708</td>
<td>70.6</td>
<td>0.94</td>
</tr>
</tbody>
</table>

a,bDifferent letters represent a significant difference (P < 0.05) between weeks.
1Values represent means.
2Area spans the granulosum, spinosum, and basal layers only.
during step-down; Hill et al., 2012; Eckert et al., 2015). One week after weaning (wk 7), calves were not able to consume enough CS to achieve ME intake equal to that of the first week of life. Because calves were unable to consume enough ME from CS, calf performance was depressed and no differences were observed in calf BW for wk 5, 6, 7, and 8. Growth depression during weaning has been reported by others in calves fed a high MR feeding rate preweaning (Cowles et al., 2006; van Niekerk et al., 2020). It is important to note that the time of weaning and the weaning method also play key roles in CS intake and performance (Meale et al., 2015; Steele et al., 2017; Welboren et al., 2019). In the current study, the calves likely performed poorly because of the early weaning age and the 1-step (decrease by 50%) weaning protocol, which is considered an abrupt weaning strategy for young calves.

Readily fermentable carbohydrates play a key role in rumen development due to SCFA production (Flatt et al., 1958; Sander et al., 1959). Total SCFA concentration was high during wk 7, which should have stimulated papillae development (Tamate et al., 1962; Stobo et al., 1966). However, during this time ME intake may have been limited to such an extent that it was

![Figure 4](image-url)  
**Figure 4.** (A) Average LPS binding protein (LBP), (B) serum amyloid A (SAA), and (C) haptoglobin (Hp) in serum from dairy calves (n = 6) fed up to 1.4 kg/d of milk replacer that were weaned during wk 6. Error bars represent SEM and arrows indicate step-down weaning. Different letters represent significant differences \((P < 0.05)\) between weeks.
dedicated solely to maintenance and was insufficient for rumen development. Based on NRC (2001), a 76.7-kg weaned calf requires 2.97 Mcal ME for maintenance; in the current study, the calves only consumed 2.33 Mcal ME at wk 7. Therefore, no changes in ruminal papillae length and surface area during weaning and up to 2 wk after weaning (wk 8) were observed.

It was unexpected that calves in the current study were ruminally acidotic (pH 5.8 for 936.3 min/d) pre-weaning (wk 5), even though CS intake was only approximately 0.130 kg/d at this time. Li et al. (2019) reported that preweaning (wk 6) mean ruminal pH was below 5.8; however, calves consumed up to 1.2 kg/d of CS during this time, which could have resulted in an accumulation of SCFA that led to low pH. The possibility exists that milk leaking into the rumen (Suárez et al., 2007) may have influenced the acidity of the rumen, especially because of the large amount of MR fed to the calves in the current study. During wk 5, papillae development was minimal, which could be attributable to the accumulation of SCFA that could not be absorbed because of reduced surface area. Rumen fermentation data were only collected starting from wk 5 onwards, but low ruminal pH and SCFA may have been present even earlier based on Rey et al. (2012). At wk 11 and 12, papillae surface area was 6.4-fold higher 6 wk after weaning (wk 12), which could have facilitated SCFA absorption leading to reduced time that ruminal pH was below 5.8. In numerical terms, acetate and propionate absorption per hour were 14.4 and 6.3% higher, respectively (Yohe et al., 2019a), and 2-dimensional papillae area was increased (Yohe et al., 2019b) in calves fed MR and CS compared with calves fed only MR at 6 wk. This finding may indicate that

**Figure 5.** A heatmap illustrating Pearson correlation coefficients between total bacteria; pH thresholds (5.2, 5.8, and 5.5); total short-chain fatty acids (SCFA); papillae surface area, length, and width; calf starter, water, and straw intakes; total protozoa; lactate; maximum (Max), mean, and minimum (Min) ruminal pH; LPS binding protein (LBP); serum amyloid A (SAA); and haptoglobin (Hp). Circles indicate significant correlations (P ≤ 0.05), with larger circles representing higher significance.
an increase in surface area may contribute to increasing SCFA absorption. However, this was not reflected during wk 11 and 12 because total SCFA concentrations were the highest during these weeks. Sampling time differences between ruminal pH (every 5 min every day) and ruminal fluid for SCFA (1 time point at the end of each week) in the present study may have contributed to the high SCFA concentrations observed because the extent of fermentation that occurred throughout the day may not have been captured. Furthermore, it is interesting that no correlation exists between the duration of pH below the thresholds and papillae size. This lack of correlation may indicate that increasing ruminal pH in calves at this age may rely on other factors, such as rumen buffering from saliva and the epithelium, instead of increased absorption of SCFA from increased surface area of papillae.

In the present study, at 3 wk after weaning, ruminal pH was below pH 5.8 for 902.26 min/d. In a study by Kim et al. (2016b), ruminal pH was below pH 5.8 for 97.6 min/d in calves fed CS and hay during wk 11 (3 wk after weaning). However, calves in the current study also had access to chopped straw, which may have decreased the time below pH 5.8 due to buffer capacity through increased rumination; however, calves only consumed 0.4 kg/d 3 wk after weaning. The variation between studies might be due to differences in calf age or CS intake, with the current study reporting 1.85 kg/d and Kim et al. (2016b) reporting 1.6 kg/d. Interestingly, Li et al. (2019) demonstrated that calves fed high-starch (42.7%, pelleted) content in CS had poor performance and reduced DMI and BW (from wk 5 onwards), compared with a CS low in starch (35.3%, whole grains), due to the depression in ruminal pH (measured at −8, −4, 0, 2, 4, 8, and 12 h relative to CS feeding once every other week). Based on these data, performance in the current study may not have been influenced by the depression in ruminal pH, as CS starch content was only 32.9%. In addition, the diet form may have also played a role in DMI in Li et al. (2019). Other studies (Kristensen et al., 2007; Laarman and Oba, 2011) did not measure pH for a long enough duration to identify if ruminal pH had an effect on intake and performance in calves during preweaning, weaning, and postweaning. However, depressed ruminal pH is well known to affect performance in lactating dairy cows, which is generally attributed to erratic and decreased DMI or fiber digestibility (Aschenbach et al., 2019).

Rumen papillae are composed of 4 cell layers, namely, the stratum corneum, granulosum, spinosum, and basal layer. In the current study, corneum thickness was highest during wk 12, which agrees with a previous study in mature animals that reported that corneum layer thickness increased with prolonged feeding of high-grain diets (Gaebel et al., 1987). However, studies also reported that high-grain diets caused corneum thickness to remain similar to that found with high-forage diets, but corneum layer sloughing increased (Steele et al., 2011, 2015). Ruminal lesions were reported in 2 groups of calves fed either a 42.7 or 35.3% starch diet at 17 wk of age; however, lesions were less severe in calves fed the lower starch diet (Gelsinger et al., 2020). The number of cells increased numerically in the current study over time, which may have resulted in an increase in corneum thickness. In the current study, when calves experienced ruminal acidosis for multiple hours per day (wk 7 and 8), no difference was observed in corneum thickness, which may indicate that depressed pH does not influence parakeratosis during this time. The granulosum thickness was unchanged in the current study, which contradicts previous reports (Steele et al., 2011; Liu et al., 2013). A high-grain diet fed to mature animals, which is similar to calves undergoing weaning, caused the granulosum layer to be reduced and compromised (Steele et al., 2011; Liu et al., 2013). This outcome negatively affects barrier function because this layer contains tight cell junction protein complexes (Kidde et al., 1951) that prevent unwanted molecules (LPS, histamine) or bacteria from translocating into the blood circulation (Owens et al., 1998; Aschenbach and Gäbel, 2000; Nagaraja et al., 2005). The sum of the spinosum and basale layer thicknesses increased from wk 5 to 8; however, previous results contradict this finding, in which cows switched from a high-forage to a high-grain diet displayed a decrease in this sum (Steele et al., 2011). In our study, a delay in differentiation of cells from the spinosum to granulosum layer may have occurred, causing the increase in thickness due to the lack of change in proliferation, as indicated by Ki67 staining between weeks. This result contrasts with the report by Yohe et al. (2019a), who found that calves fed CS had greater proliferation and differentiation in the spinosum and basale layer, compared with calves fed only MR.

Ruminal pH is closely associated with rumen epithelium barrier integrity in mature ruminant experimental models (Penner et al., 2010), but this association is largely unknown in dairy calves. However, ruminal pH is not the only factor affecting ruminal barrier integrity. Low ruminal pH in conjunction with high SCFA concentration can have a significant effect on barrier function and epithelial integrity ex vivo (Meissner et al., 2017). The translocation of unwanted molecules (LPS, histamine) or bacteria into the bloodstream will activate an inflammatory response (Khafipour et al., 2009a). Although SAA increased numerically during wk 7, the ruminal acidosis experienced during wk 5 to 8 did not seem to influence LBP, SAA, or Hp. This
finding may indicate that the barrier function during this time was not affected because the cornu and the granulosum were not affected during these weeks. A moderate negative correlation existed between CS intake and SAA, suggesting that increased CS intake did not evoke an inflammatory response in the current study. This result contrasts with previous findings of increased blood concentrations of LBP, SAA, and Hp in cows fed high-grain diets (Khafipour et al., 2009a), indicating inflammatory response activation. Most of the current knowledge regarding ruminal pH and its effect on rumen and animal health pertains to adult animals, and most studies assume that this knowledge also applies to calves; however, the data observed in the current study suggest that this assumption might not be accurate. The rumen environment was acidic during wk 7 may be an indication of weaning stress experienced by calves, as Kim et al. (2011) reported previously (Orro, 2008; Tóthová et al., 2015). Yet SAA and Hp decreased thereafter to 80 to 200 µg/mL and 100 to 200 µg/mL, respectively, which are considered baseline levels for healthy calves (Seppä-Lassila et al., 2013). A similar pattern was observed in the current study. The peak at approximately the first week of life has been suggested to occur due to acute-phase proteins possibly being transferred through colostrum intake and entering calf circulation (McDonald et al., 2001). Another possibility may be that proinflammatory cytokines, such as IL-6, are ingested via colostrum and stimulate acute-phase protein production in the liver (Hagiwara et al., 2000). The numerical increase of SAA during wk 7 may be an indication of weaning stress experienced by calves, as Kim et al. (2011) attributed elevated SAA concentrations 3 and 5 d after weaning to weaning stress. In conclusion, further research regarding the effect of depressed ruminal pH in calves during weaning on local inflammation and tissue integrity in the rumen is warranted.

A peak in acute-phase proteins (SAA and Hp) was observed around 1 wk of life in calves and has been reported previously (Orro, 2008; Tóthová et al., 2015). Yet SAA and Hp decreased thereafter to 80 to 200 µg/mL and 100 to 200 µg/mL, respectively, which are considered baseline levels for healthy calves (Seppä-Lassila et al., 2013). A similar pattern was observed in the current study. The peak at approximately the first week of life has been suggested to occur due to acute-phase proteins possibly being transferred through colostrum intake and entering calf circulation (McDonald et al., 2001). Another possibility may be that proinflammatory cytokines, such as IL-6, are ingested via colostrum and stimulate acute-phase protein production in the liver (Hagiwara et al., 2000). The numerical increase of SAA during wk 7 may be an indication of weaning stress experienced by calves, as Kim et al. (2011) attributed elevated SAA concentrations 3 and 5 d after weaning to weaning stress. In conclusion, further research regarding the effect of depressed ruminal pH in calves during weaning on local inflammation and tissue integrity in the rumen is warranted.

CONCLUSIONS

To our knowledge, this study is the first to investigate ruminal pH up to 6 wk after weaning. We hypothesized that rumen structural development is delayed up to 2 wk after weaning, which causes a depression in ruminal pH during this time and subsequently leads to an acute inflammatory response. Metabolizable energy supply from CS may have an effect on rumen development, as it took several weeks for rumen papillae surface area to increase. Ruminal pH is depressed even before step-down weaning in calves with low starter intake, which may be due to reduced buffer capacity. It took several weeks for the rumen to adapt so as to not be in a severe state of ruminal acidosis after weaning. The prolonged decreased pH may be caused by a combination of delayed rumen development and ability to buffer the rumen after weaning. Barrier integrity was likely not affected by the prolonged pH depression and increased SCFA because papillae strata and sloughing scores were largely unaffected. Furthermore, acute-phase proteins (LBP, SAA, and Hp) were not influenced during this time, indicating that no inflammatory response was occurring. Further investigation is warranted to determine if ruminal acidosis is harmful during the pre- and postweaning periods in calves as it is in mature ruminants.

ACKNOWLEDGMENTS

The authors thank the Natural Sciences and Engineering Research Council of Canada, Alberta Milk (Edmonton, AB, Canada), Lallemand (Montreal, QC, Canada), Westgen (Abbotsford, BC, Canada), BC Dairy Association (Burnaby, BC, Canada), SaskMilk (Regina, SK, Canada), and the Dairy Farmers of Manitoba (Winnipeg, MB, Canada) for the financial support provided for this study. The authors also thank B. Tehr (University of Alberta, Edmonton, AB, Canada) for assistance with calf management. The authors have not stated any conflicts of interest.

REFERENCES


Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2007. Sever-
ity of ruminal acidosis in primiparous Holstein cows during the per-
3168/jds.2007-0302(07)2638-3.

Penner, G. B., M. Oba, G. Gäbel, and J. R. Aschenbach. 2010. A
single mild episode of subacute ruminal acidosis does not affect
ruminal barrier function in the short term. J. Dairy Sci. 93:4838–

Rey, M., F. Enjalbert, and V. Monteils. 2012. Establishment of rumi-
nal enzyme activities and fermentation capacity in dairy calves from birth through weaning. J. Dairy Sci. 95:1500–1512. https://

Saleem, F., S. Bouatra, A. C. Guo, N. Psychogios, R. Mandal, S. M.
Dunn, B. N. Ametaj, and D. S. Wishart. 2013. The bovine rumi-
1007/s13006-012-0458-9.

The stimulatory effect of sodium butyrate and sodium propion-
one on the development of rumen mucosa in the young calf.
0302(59)00772-6.


Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plai-
zier, and B. W. McBride. 2011. Bovine rumen epithelium under-
goes rapid structural adaptations during grain-induced subacute

Steele, M. A., J. H. Doelman, L. N. Leal, F. Soberon, M. Carson, and
J. A. Metcalf. 2017. Abrupt weaning reduces postweaning growth and is associated with alterations in gastrointestinal markers of
development in dairy calves fed an elevated plane of nutrition dur-
doi.org/10.3168/jds.2016-12310.

Steele, M. A., C. Schiestel, O. AlZahal, L. Dionissopoulos, A. H. Laar-
man, J. C. Matthews, and B. W. McBride. 2015. The periparturi-
ent period is associated with structural and transcriptomic adapta-

and low abundance of classical ruminal bacterial species in the
bovine rumen revealed by relative quantification real-time PCR.
S00253-006-0902-v.

calf. 1. The effect of diets containing different proportions of
188. https://doi.org/10.1017/S000711456600021.

Suárez, B. J., C. G. Van Reenen, N. Stockhole, J. Dijkstra, and W. J.
J Gerrits. 2007. Effect of roughage source and roughage to con-
centrate ratio on animal performance and rumen development in
JDS.2006-524.

2004. Development of an assay to quantify rumen ciliate protozoal

Effect of various diets on the anatomical development of the
3168/jds.S0022-0302(62)9406-5.

Tóthova, C., O. Nagy, V. Nagyová, and G. Koviáč. 2015. Changes in
the concentrations of acute phase proteins in calves during the first
1517/avc-2015-0022.

van Niekert, J. K., A. J. Fischer-Trustos, L. L. Deikun, J. D. Quig-
ley, T. S. Dennis, F. X. Suarez-Mena, T. M. Hill, R. L. Schlot-
terbeck, L. L. Guan, and M. A. Steele. 2020. Effect of amount of
milk replacer fed and the processing of corn in starter on growth
performance, nutrient digestibility, and rumen and fecal fibrolib-

van Niekert, J. K., M. Middeldorp, and M. A. Steele. 2018. Techni-
cal note: The development of a methodology for ruminal and col-
on tissue biopsying of young Holstein dairy calves. J. Dairy Sci.

dietary fiber, neutral detergent fiber, and nonstarch polysaccha-

Welboren, A. C., L. N. Leal, M. A. Steele, M. A. Khan, and J. Martín-
teres. 2019. Performance of ad libitum fed dairy calves weaned
 doi.org/10.1002/a/acv.196001818.

Wood, K. M., S. I. Palmer, M. A. Steele, J. A. Metcalf, and G. B.
Penner. 2015. The influence of age and weaning on permeability of
the gastrointestinal tract in Holstein bull calves. J. Dairy Sci.

Yoh, T. T., H. Schramm, C. Parsons, H. Tucker, B. D. Enger, N. R.
Hardy, and K. M. Daniels. 2019a. Form of calf diet and the rumen.

Yoh, T. T., H. Schramm, R. R. White, M. D. Hanigan, C. Parsons,
Form of calf diet and the rumen. II: Impact on volatile fatty acid
JDS.2019-16450.

Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality
community DNA from digesta and fecal samples. Biotechniques

ORCIDS

J. K. van Niekert https://orcid.org/0000-0003-3955-6850
M. Middeldorp https://orcid.org/0000-0003-1740-7942
L. L. Guan https://orcid.org/0000-0002-8140-4371
M. A. Steele https://orcid.org/0000-0001-6941-6205