J. Dairy Sci. 105:1542–1554
https://doi.org/10.3168/jds.2021-20939

Feeding an acetate-based oral electrolyte reduces the ex vivo *Escherichia coli* growth potential in the abomasum of calves fed oral electrolytes alone or 30 minutes following a milk feeding compared with feeding a bicarbonate-based oral electrolyte

B. A. Kasl,[1] V. S. Machado,[1] M. T. Henniger,[2] P. R. Myer,[2] and M. A. Ballou[1]*
[1]Department of Veterinary Sciences, Texas Tech University, Lubbock 79409
[2]Department of Animal Science, University of Tennessee, Knoxville 37996

ABSTRACT

Oral electrolyte solutions (OES) are a common, on-farm therapy to reestablish hydration and electrolyte balances in scouring and stressed calves. The objectives were to determine the effects of OES alkalinizing agent and the presence of a milk replacer feeding before OES administration on the abomasal environment in healthy Holstein calves. Abomasum cannulation was performed on 16 Holstein bull calves at 5 d of age. One calf was removed from the study before the calves were randomly assigned to treatments at 9 d of age. Treatments were arranged as a 2-by-2 factorial, with the following factors: oral electrolyte alkalinizing agent [acetate (A) or bicarbonate (B)] and liquid meal type milk replacer (MR) + OES (MR-A, MR-B), or OES only (OES-A, OES-B)]. The OES differed only by alkalinizing agent. On d 9, calves assigned to MR-A (n = 4) or MR-B (n = 4) received their morning MR aliquot 0.5 h before feeding 2 L of OES; the OES-A (n = 3) and OES-B (n = 4) treatment groups were fed 2 L of OES only. Peripheral blood samples and postprandial abomasal fluid samples were collected to assess abomasal pH, abomasal emptying rate (AER), and ex vivo abomasal *Escherichia coli* growth potential. Postprandial pH was greater in calves fed MR or B-based OES. Abomasal emptying rate was slower in calves receiving MR + OES, regardless of the alkalinizing agent. Ex vivo *E. coli* colony-forming unit counts were greater in calves fed either MR + OES or bicarbonate-based OES. Supplementing bicarbonate OES in addition to MR alters abomasal dynamics and may promote *E. coli* growth in postprandial abomasal fluid, partially due to sustained elevations in gastric pH and delayed gastric emptying rates. The OES containing sodium acetate limited ex vivo *E. coli* growth potential in abomasal fluid, thereby potentially reducing the risk of additional enteric bacterial complications associated with OES therapy.

Key words: abomasum, calf, oral electrolyte solutions

INTRODUCTION

Calf morbidity and mortality associated with gastrointestinal disease continues to plague the dairy industry. Recent nationwide surveys indicated a 33.9% morbidity risk and 5.0% mortality risk in heifer populations, with the greatest proportion of adverse health events occurring within the first 21 d of age (Urie et al., 2018). As neonates, calves have a total body water content of greater than 70% of total BW until about 40 d of age. Their significant body water percentage, coupled with severe extracellular fluid losses during periods of environmental stress or gastrointestinal disease, makes calves highly susceptible to dehydration and its related sequelae (Wickramasinghe et al., 2019). Implementation of practical, effective treatments to offset body water losses via electrolyte therapies has improved clinical outcomes in sick calves (Smith, 2009).

Oral electrolyte solutions (**OES**) are a mainstay of economical, on-farm treatments to restore hydration and acid-base balance in stressed or scouring calves. Generally, the composition of OES consist of physiologically relevant cations (e.g., sodium and potassium), anions (e.g., chloride), an alkalinizing agent, and a carbohydrate source (e.g., dextrose, maltodextrin). Sodium bicarbonate, acetate, or propionate are commonly incorporated into oral electrolyte formulations as alkalinizing agents to correct acidemia and strong ion acidosis (e.g., hyponatremia, hyperchloremia, and hyper-D-lactatemia; Schwedhelm et al., 2013; Smith, 2009). The OES containing sodium bicarbonate effectively treat metabolic acidosis; however, they result in sustained increases in abomasal pH (Marshall et al., 2008). Temporarily reducing the acidity of the

Received June 29, 2021.
Accepted October 21, 2021.
*Corresponding author: michael.ballou@ttu.edu

abomasal compartment may create opportunities for potential enteric pathogenic bacterial overgrowth to occur. Prolonged increases in abomasal pH were not observed in calves supplemented with OES containing acetate (Smith et al., 2012). Regeneration of damaged gastrointestinal epithelium following pathogenic insult requires a sufficient nutritional supply of energy to turn over intestinal stem cells (Chen et al., 2019). Because the caloric density of OES is not sufficient to meet daily energetic demands, continued routine administration of milk meals supports the requirements for maintenance, preservation of intestinal epithelial integrity, and growth.

Management of dehydrated calves commonly involves administration of an OES, usually several hours following a milk meal. However, calf-raising operations may supplement OES soon after a milk meal due to labor and managerial restraints (Smith and Berchtold, 2014). Large volumes or osmotically dense solutions administered at a single feeding or within a short duration of time may slow abomasal emptying rates (**AER**; Nouri and Constable, 2006). Reduced rates of abomasal emptying were associated with a multitude of gastroenteric diseases, including abomasitis, bloat, and malabsorptive diarrhea (Burgstaller et al., 2017). The complex relationship between AER, abomasal disease, and the abomasal microbiome remains largely unknown.

The objectives of this experiment were to determine the effects oral electrolyte alkalinizing agent type and the presence or absence of a milk meal 30 min before OES supplementation on abomasal dynamics and the gastric bacterial communities in Holstein calves less than 14 d of age. The hypothesis was that feeding an OES formulated with acetate as the buffer would decrease the pH in the abomasum and reduce the ability of *Escherichia coli* to grow and survive in fluid collected from the abomasum when compared with feeding an OES formulated with bicarbonate. Further, this effect of acetate would be independent of when the OES was fed relative to the previous milk replacer (**MR**) feeding.

MATERIALS AND METHODS

Animals, Housing, and Management

All experimental procedures were approved and in compliance with the Livestock Issues and Research Unit (LIRU) of the USDA-ARS Institutional Animal Care and Use Committee, protocol # LIRU-1912F. The experiment was conducted during September 2019. Sixteen Holstein bull calves (2 ± 1 d of age) were transported 85 km from a commercial dairy farm to the LIRU in New Deal, Texas. Approximately 3.8 L of pooled colostrum was administered via esophageal feeder within the first 8 h of life on-farm. Upon arrival, individual BW and peripheral venous blood samples were obtained (6-mL no additive Vacutainer, Becton, Dickinson). Serum was isolated from each blood sample following centrifugation (1,500 × *g* for 5 min at 23°C) and total serum protein assessed using a handheld refractometer (Atago). All calves had a serum total protein value ≥5.5 g/dL (mean ± SD; 6.2 ± 0.4 g/dl), indicative of adequate passive transfer of immunity (Weaver et al., 2000). Calves were housed individually indoors on slatted flooring (Tenderfoot; Tandem Products) within polyethylene calf pens (1.9 m × 1.1 m; Agri-Plastics). Physical contact was not permissible between animals in adjacent pens due to the structure of the individual hutches. Environmental temperatures and relative humidity were measured hourly using a wireless data logger (Kestrel DROP D2). Thermoneutral ambient temperatures (22.7–24.6°C) were maintained throughout the experimental period via HVAC and positive pressure tube ventilation systems.

All calves were fed 455 g/d (as-fed) of a nonmedicated, 22% CP − 20% fat (DM basis; Table 1) commercially available MR (MB Nutritional Sciences). The MR powder was reconstituted to a total volume of 3.8 L with hot water (10.7% TS). The final MR solution temperature measured between 38.3 to 40.8°C at the time of each feeding. Calves were individually bottle-fed twice daily at 0630 and 1630 h in equal aliquots (1.9 L/milk meal), beginning with the afternoon feeding on d 0 of the experiment. Refusals were measured immediately following each mealtime. Access to a texturized calf starter (Table 1) and fresh water were available ad libitum throughout the entire experiment. The calf starter and water were replaced daily.

Abomasum Cannulation Surgery

Immediately following the afternoon MR feeding on d 4, calf starter and water were removed from all calves. On d 5, the morning MR feeding was withheld to ensure a total fasting time of at least 12 h before anesthetic induction. Perioperative protocols and surgical placement of an indwelling abomasal cannula were adapted from previously described procedures by Ahmed et al. (2005). Briefly, general anesthesia was achieved by intramuscular administration of xylazine (Rompun, Bayer Healthcare, LLC; 0.5 mg/kg) and ketamine (Zetamine, VETone; 2 mg/kg). Each calf was placed in left lateral recumbency. The right paracostal and paralumbar regions were clipped and aseptically prepared. A 17-cm vertical skin incision was made approximately 5 cm caudoventral to the last rib. The underlying fascia and skeletal muscles were transected, and parietal peritoneum incised to access the abdominal cavity. A brief abdomi-

nal exploration ensued. The abomasum was located, isolated, and exteriorized. Two concentric purse-string sutures (2- and 3-cm diameter) were placed within the ventrolateral aspect of the abomasal body (corpus abomasi) wall using 2–0 polydioxanone (PDS II, Ethicon Inc.). Within the preplaced concentric sutures, a stab incision was made into the abomasal lumen. A sample of abomasal fluid (approximately 3 mL) was collected aseptically using an 8 French polypropylene catheter (Cardinal Health). Abomasal fluid samples were immediately preserved at −80°C for subsequent analyses. Following abomasal fluid collection, a 20 Fr Percutaneous Endoscopic Gastrostomy tube (MILA Inc.) with an internal diameter of 5 mm, external diameter of 7 mm, and collapsible bumper was guided through the stab incision. The purse-string sutures were tightened to secure the gastrostomy tube within the abomasal lumen. The serosal surface of the abomasum and abdominal cavity were lavaged with sterile 0.9% NaCl solution (VETone). The gastrostomy tubing was exteriorized through a stab incision in the abdominal wall 3-cm dorsocaudal to the initial incision. Sutures were placed within the gastric wall to secure the abomasum against the parietal peritoneum; thereafter, the body wall was closed routinely. The gastrostomy tube was adhered to the skin of the lateral abdomen using butterfly tape (Johnson & Johnson Medical, Division of Ethicon, Inc.) and nonabsorbable suture (2–0 Ethilon, Ethicon, LLC). Anesthetic reversal agent (tolazoline; Tolazine; Akorn Animal Health, Inc.; 3.5 mg/kg i.m.), antibiotics (ceftiofur sodium; Naxcel; Zoetis; 2.2 mg/kg s.c.), and flunixin meglumine (Prevail; VETone; 1 mg/kg i.v.) were administered during the postoperative recovery period. Each calf was fitted with a compression tube (Weaver Leather, LLC) to reduce the risk of a calf pulling the gastrostomy tubing out of the abomasum. Postoperative recovery occurred uneventfully in all calves. In replace of the afternoon MR feeding on d 5, 2 L of the acetate-based OES (Table 2) was offered in addition to ad libitum fresh water and calf starter. The MR feedings resumed the morning of d 6 as previously described. All calves had 4 d to recover from cannulation surgery before experimental treatment and data collection. One calf developed acute septicemic events the day following the cannulation procedure and was removed from the study.

Table 1. The formulated nutrient contents of the milk replacer and the calf starter fed to preweaning Holstein bull calves

Nutrient	Milk replacer[1]	Calf starter[2]
DM, %	95.7	88.7
CP, %	22	20
Ether extract, %	20	2.5
Crude fiber, %	—	10
ADF (max), %	0.15	12
ME, Mcal/kg	4.65	3.08
Calcium, %	1.25	1.2
Phosphorus, %	0.7	0.45
Selenium, ppm	—	0.3
Vitamin A (min), IU/kg	13,636	2,955
Vitamin D3 (min), IU/kg	4,545	—
Vitamin E (min), IU/kg	45.5	—

[1]Nonmedicated milk replacer was formulated by MB Nutritional Sciences (MB Nutritional Sciences, LLC) and fed to all enrolled animals throughout the experimental period. Ingredients included: dried whey, dried whey protein concentrate, dried whey product, animal and vegetable fat (preserved with butylated hydroxyanisole and butylated hydroxytoluene), dried skim milk, lecithin, dicalcium phosphate, calcium carbonate, citric acid (preservative), L-lysine monohydrochloride, DL-methionine, vitamin A supplement, vitamin D supplement, vitamin E supplement, ascorbic acid, magnesium oxide, zinc sulfate, ferrous sulfate, niacin supplement, manganese sulfate, calcium pantothenate, vitamin B_{12} supplement, thiamine mononitrate, riboflavin supplement, copper sulfate, pyridoxine hydrochloride, ethylenediamine dihydroiodide, folic acid, choline chloride, cobalt sulfate, selenium yeast, sodium silico aluminate, mono and diglycerides of edible fats or oils, artificial flavor.

[2]Medicated, texturized complete calf starter formulated by MB Nutritional Sciences LLC and manufactured by Hi-Pro Feeds was formulated with monensin at 50 ppm. Ingredients included whole corn, cracked corn, soybean meal, canola meal, rolled oats, wheat midds, dried distillers grains, calcium carbonate, salt, yeast extract, zinc amino acid complex, zinc sulfate, manganese amino acid complex, manganese sulfate, copper amino acid complex, cobalt glucoheptonate, vitamin E supplement, vitamin A supplement, vitamin D supplement, selenium yeast, ethylenediamine dihydroiodide, and natural and artificial flavors.

Experimental Design and Treatment Allocation

The experimental design was a 2-by-2 factorial randomized incomplete block study design. Immediately following the afternoon MR feeding on d 8, the 15 remaining calves were blocked by BW into 4 blocks and then randomly assigned to 1 of 4 treatments within

Table 2. Composition of the 2 oral electrolyte solutions (OES), differing only by alkalinizing agent (sodium acetate, sodium bicarbonate), supplemented to Holstein calves either with or without a previous milk meal[1]

Nutrient	Sodium acetate OES	Sodium bicarbonate OES
Sodium (Na^+), mmol/L	100	100
Potassium (K^+), mmol/L	25	25
Chloride (Cl^-), mmol/L	45	45
Calcium (Ca^{2+}), mmol/L	0	0
Sodium acetate, m*M*	80	0
Sodium bicarbonate, m*M*	0	80
Glucose, mmol/L	100	100
Strong ion difference,[2] mEq/L	80	80
Osmolality,[3] mOsm/kg	350	350

[1]Both oral electrolyte solutions were prepared by M. A. Ballou.

[2]Effective strong ion difference = $[Na^+] + [K^+] - [Cl^-]$.

[3]Osmolality (mOsm/kg) was determined by summing the osmolality of carbohydrates (glucose) and minerals (Na^+, K^+, Cl^-, and Ca^{2+}).

each BW block. The greatest BW block was the incomplete block. The main effects were oral electrolyte alkalinizing agent [sodium acetate (A) or sodium bicarbonate (B)] and liquid meal type (MR + OES or OES only). The 4 dietary treatment groups were as follows: (1) previous MR meal + OES meal containing sodium acetate (MR-A; n = 4); (2) previous MR meal + OES meal containing sodium bicarbonate (MR-B; n = 4); (3) OES meal containing sodium acetate only (OES-A; n = 3); and (4) OES meal containing sodium bicarbonate only (OES-B; n = 4). Calves assigned to either MR-A or MR-B received MR at 0630 followed by OES at 0700 h. Calves assigned to OES-A or OES-B only received their assigned OES at 0700 h, the previous MR meal was on d 8 at 1630 h. Each OES had similar composition and osmolarity (350 mOsm/L), differing only by alkalinizing agent (80 m*M* of either A or B; Table 2). Both OES were prepared by MB Nutritional Sciences (Lubbock, TX). The OES were offered using a nipple bottle, similar to the MR feedings. A final sample size of n = 3 was based upon effect size reductions of the main effect of OES type of (1) 1.5 logarithmic base 10 with a standard deviation of 0.65 in *E. coli* counts in abomasal fluid that were spiked ex vivo with *E. coli* and (2) abomasal peak pH from 7 to 5.5 with a standard deviation of 0.6 with 5% and 20% protections against Type 1 and 2 errors, respectively. Sixteen calves were surgically fit with abomasal cannulas to ensure we had at least n = 3 if any calf died or had to be removed due to complications with the abomasal cannulation surgery. M.A. Ballou randomized calves to treatments and administered the treatments. B.A. Kasl remained blinded to treatments because he was responsible for assessing all outcome variables.

Data and Sample Collection

Calf BW was measured on arrival and on d 9. Acetaminophen (50 mg/kg; 4-acetamidophenol; ACROS Organics) was mixed into each OES on d 9 to indirectly assess AER. Jugular venous blood samples (6 mL potassium-citrate, Vacutainer, Becton, Dickinson and Company) were collected −0.5, 0, 1, 2, 3, 4, 6, and 8 h relative to the OES administration at 0700 h. Following centrifugation (1,500 × *g* for 15 min at 23°C), plasma was isolated and stored at −80°C for later analyses. Aseptic abomasal fluid sampling occurred at −0.5, 0, 1, 2, 4, and 6 h after OES supplementation. Volume and pH (Orion Star A111 pH Benchtop Meter, ThermoFisher Scientific) were measured immediately following collection. A sample from 6 h post-OES supplementation was immediately stored at −80°C for subsequent analysis of bacterial communities.

Plasma Acetaminophen Quantification

Acetaminophen absorption kinetics were validated and frequently cited as a practical, indirect assessment of AER in preweaning calves (Burgstaller et al., 2017). Plasma samples were thawed at 22°C for spectrophotometric analyses. Acetaminophen concentrations were quantified in duplicate using a commercially available colorimetric kit (Paracetamol Assay Kit, K8002). The assay was performed as instructed by the supplier and optimized for reduced sample volumes. The intra- and interassay coefficients of variation were 2.7 and 3.2%, respectively.

Ex Vivo Bacteriologic Growth Potential

On d 8, a purified *E. coli* isolate (ATCC #8739) was cultured overnight in tryptic soy broth (**TSB**) at 37°C. On d 9, the overnight culture was stored at 4°C and 4 h before each abomasal fluid collection, 100 μL of the overnight culture was subcultured into 20 mL of fresh TSB and incubated for 4 h at 37°C. Preliminary data indicated an incubation period of 4 to 6 h would achieve approximately 5×10^8 cfu/mL. A 1:100 dilution of the isolate was then performed using sterile 1× PBS. A 1-mL subsample of abomasal fluid from each time point was inoculated with 10 μL of the diluted *E. coli* culture. The inoculated abomasal fluid cultures were aerobically incubated for 4 h at 37°C. Following the incubation, cfu/mL was quantified using xylose lysine deoxycholate agar (HiMedia Laboratories). Each subculture that was used to inoculate the abomasal cultures at each time was serially diluted and plated to determine the colony-forming units that were inoculated at each time.

Abomasum Fluid Bacterial Communities

The 16S rRNA gene analyses were performed on abomasal fluid samples collected intraoperatively during abomasal cannulation (d 5) and at the 6 h post-OES feeding time point on d 9. The 6 h post-OES time point was selected for abomasal fluid sampling to determine any persistent postprandial shifts in the abomasal bacterial communities. Samples were thawed at room temperature and immediately processed. To maximize yields, each sample was centrifugated (13,000 × *g* for 5 min at 4°C) and the supernatant discarded. Bacterial DNA within approximately 250 mg of pelleted biosolids was isolated using a commercially available kit (QIAamp PowerFecal DNA Kit). Microbial cells were lysed via bead-beating mechanical disruption and DNA subsequently eluted through a silica membrane within a spin column. The DNA yield and purity were evalu-

ated using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific). The ratio of absorbances at 260 nm and 280 nm were calculated to be 1.80 or greater in all samples, thus considered to be of acceptable purity for further downstream analyses.

Bacterial DNA was sequenced at the Center for Biotechnology and Genomics at Texas Tech University (Lubbock, TX). The 16S rDNA amplicon sequencing library preparation procedures as outlined by Illumina using the MiSeq system. Briefly, the V3-V4 hypervariable regions of the bacterial 16S rRNA were targeted and amplified using PCR with universal primers. Library samples were normalized, pooled, and sequenced using an Illumina MiSeq (Illumina).

The Illumina MiSeq 2x250 FASTQ sequence files were imported into R for analysis of bacterial communities using a pipeline as described by Callahan et al. (2016b). Open-source R packages 'phyloseq' (McMurdie and Holmes, 2013) and 'DADA2' (Callahan et al., 2016a) were used to perform filtering, merging, and taxonomic assignment. Forward and reverse reads were trimmed based on quality score (Q ≥25) and expected errors per read were set to 2 for forward reads and 4 for reverse reads, filtering out any data not meeting these criteria (Edgar and Flyvbjerg, 2015). Following, Divisive Amplicon Denoising Algorithm 2 (**DADA2**) was used to identify amplicon variants while correcting for amplicon errors using a naïve Bayesian classifier (Callahan et al., 2016a; Wang et al., 2007). From DADA2, amplicon sequence variants (**ASV**) were generated (Callahan et al., 2017; Glassman & Martiny, 2018). Quality-filtered forward and reverse reads were then merged and chimeras were removed. Merged reads then underwent taxonomic assignment to the genus level using the SILVA v132 database (Quast et al., 2013). The singletons and cyanobacteria were removed from the data.

Statistical Analysis

Continuous, repeatedly measured data were analyzed using restricted-maximum likelihood ANOVA via PROC MIXED in SAS v. 9.4 (SAS Institute Inc.). The linear mixed model included fixed effects of OES alkalinizing agent, liquid meal type, time, and their interactions. The model also included a random effect of BW block. The subject of the repeated statement was calf nested within treatment combination. The model was evaluated using all applicable covariance structures with unequal spacing. Analysis for each variable used the covariance structure with the lowest Bayesian information criterion.

Differences in minimal postprandial abomasal pH, maximal postprandial abomasal pH, AUC_{480} abomasal pH were analyzed as a 2-way ANOVA with the main fixed effects of OES alkalinizing agent and fluid meal type and the random effect of BW block using PROC MIXED. Further, the AER was assessed using methodologies previously described (Marshall et al., 2005; MacPherson et al., 2016). Maximal acetaminophen concentration (C_{max}), time to maximal acetaminophen concentration (T_{max}), and AUC_{510} were analyzed as a 2-way ANOVA with the main fixed effects of OES alkalinizing agent and fluid meal type and the random effect of BW block using PROC MIXED. Differences of $P \leq 0.05$ were considered significant and tendencies were considered when $0.10 \leq P > 0.05$. Significant treatment by time differences were further evaluated at each time point using sliced treatment differences with a Duncan correction to control family-wise Type 1 error rates. Data are reported as least squares means ± standard error of the mean.

Alpha- and β-diversity metrics were measured in R. For α diversity, microbial community diversity parameters of estimated richness (Chao1) and richness and evenness (Shannon) were analyzed. Differences among treatments for α diversity were tested in SAS. Histograms were visually assessed and a Shapiro-Wilk score of ≥ 0.9 was used to determine normality. Non-normal data were log-transformed. Non-normal data that were log-transformed and contained multiple instances of 0 abundances for a specific microbial community were then assigned a value of 0.00001. A mixed model ANOVA was used with least squares means at a statistical significance of $P \leq 0.05$. Alpha diversity, defined as the microbial richness and evenness within individual samples, was assessed using predictive indices (Chao1 richness, Shannon's diversity index) and observed richness. Beta diversity was measured using a Bray-Curtis distance matrix producing a principal coordinate analysis (**PCoA**). A PERMANOVA was conducted with 999 permutations to determine differences among treatments for the PCoA using package 'vegan' in R (Oksanen et al., 2013). Differential abundances among treatments were analyzed in SAS v9.4 using a mixed model ANOVA to determine the fixed effects of liquid meal composition, type of OES alkalinizing agent, and the interaction of liquid meal composition by OES alkalinizing agent. The model also include a random effect of BW block. Differences in least squares means were separated with Fisher's least significant differences, with significance determined by $P \leq 0.05$.

RESULTS

Total serum protein at enrollment, initial BW, and BW on d 9 did not differ among treatments ($P \geq 0.675$). All abomasal cannulas remained intact and patent following surgical placement through the end of the

observation period. All calves voluntarily ingested the entirety of their dietary treatments within 5 min.

Abomasal pH

Abomasal pH data are reported in Table 3 and Figure 1. There was a tendency for an interaction between OES alkalinizing agent × fluid meal type × time ($P = 0.081$). Further, there were OES alkalinizing agent × time and fluid meal type × time interactions ($P \leq 0.003$). There was an OES alkalizing agent × fluid meal type interaction at 1 h following OES consumption, whereas calves fed OES-B had greater pH than OES-A, but MR-B and MR-A were not different from each other. Calves fed both MR and OES had greater pH at 0, 2, 4, and 6 h relative to OES only consumption.

Calves fed a MR meal before OES supplementation had greater mean postprandial abomasal pH values, regardless of the type of OES administered ($P < 0.001$). The OES alkalinizing agent did not affect mean postprandial pH when MR was administered before OES supplementation ($P = 0.635$). Minimum postprandial abomasal pH was similar across treatments ($P \geq 0.101$). There was a tendency for an OES alkalinizing agent × fluid meal type interaction for maximum abomasal pH ($P = 0.056$); calves fed either MR or a bicarbonate-based OES had greater maximum pH compared with calves fed OES only or acetate-based OES. Calves administered MR before OES supplementation had a greater AUC_{510} ($P < 0.001$).

Abomasal Emptying Rate

Indirect assessment of AER was achieved by measuring plasma acetaminophen concentration following its consumption within the supplemented OES. Acetaminophen absorption data as indices of AER are presented in Table 4. Maximal acetaminophen concentration ($\mathbf{C_{max}}$) and time to maximal acetaminophen concentration ($\mathbf{T_{max}}$) values are dependent on the rate of gastric emptying thus may be used to assess AER in calves (Marshall et al., 2005; Burgstaller et al., 2017). The T_{max} was prolonged in calves fed MR in addition to an OES ($P = 0.004$). There was also a fluid meal type main effect difference for C_{max} ($P = 0.008$). The OES-B treated calves had a greater C_{max} compared with calves fed either MR-A or MR-B diets ($P \leq 0.030$).

Ex Vivo E. coli Growth Potential

Ex vivo E. coli growth potential is presented in Figure 2. There was no interaction of OES alkalizing agent × fluid meal type × time interaction ($P = 0.206$);

Table 3. Summary of abomasal luminal pH data of Holstein bull calves supplemented with oral electrolyte solutions (OES) containing different alkalinizing agents (sodium acetate, sodium bicarbonate) either with or without a prior milk replacer (MR) feeding; data are presented as mean ± largest SEM

	Treatment[1]					Fixed effect $P \leq$						
Item	OES-A	OES-B	MR-A	MR-B	Largest SEM	OES	MR	Time	OES × MR	OES × Time	MR × Time	OES × MR × Time
Mean pH	2.41	2.83	3.93	4.07	0.23	0.208	0.001	0.001	0.515	0.003	0.001	0.081
Postprandial pH, min	1.6	1.8	1.91	2.17	0.21	0.253	0.101	—	0.882	—	—	—
Postprandial pH, max	4.82[a]	7.17[c]	5.84[ab]	6.40[bc]	0.47	0.005	0.773	—	0.056	—	—	—
AUC_{510}[2]	1,254[a]	1,429[a]	2,080[b]	2,133[b]	120.9	0.319	0.001	—	0.585	—	—	—

[a–c]Data values within a row that have differing superscript letters or symbols are significantly different ($P < 0.05$).

[1]Calves were randomly assigned to 1 of 4 dietary treatments that differed by the presence or lack of a milk meal (225 g of as-fed of 22% CP − 20% fat MR at 10.7% solids) administered 30 min before OES supplementation containing different alkalinizing agents: OES-A and OES-B treatment groups received only 2 L of OES containing either 80 mmol/L of either sodium acetate or sodium bicarbonate, respectively; MR-A and MR-B treatment groups received MR and OES containing 80 mmol/L of either sodium acetate or sodium bicarbonate, respectively.

[2]Area under the curve for 510 min (30 min prior to feeding OES through 8 h after OES feeding).

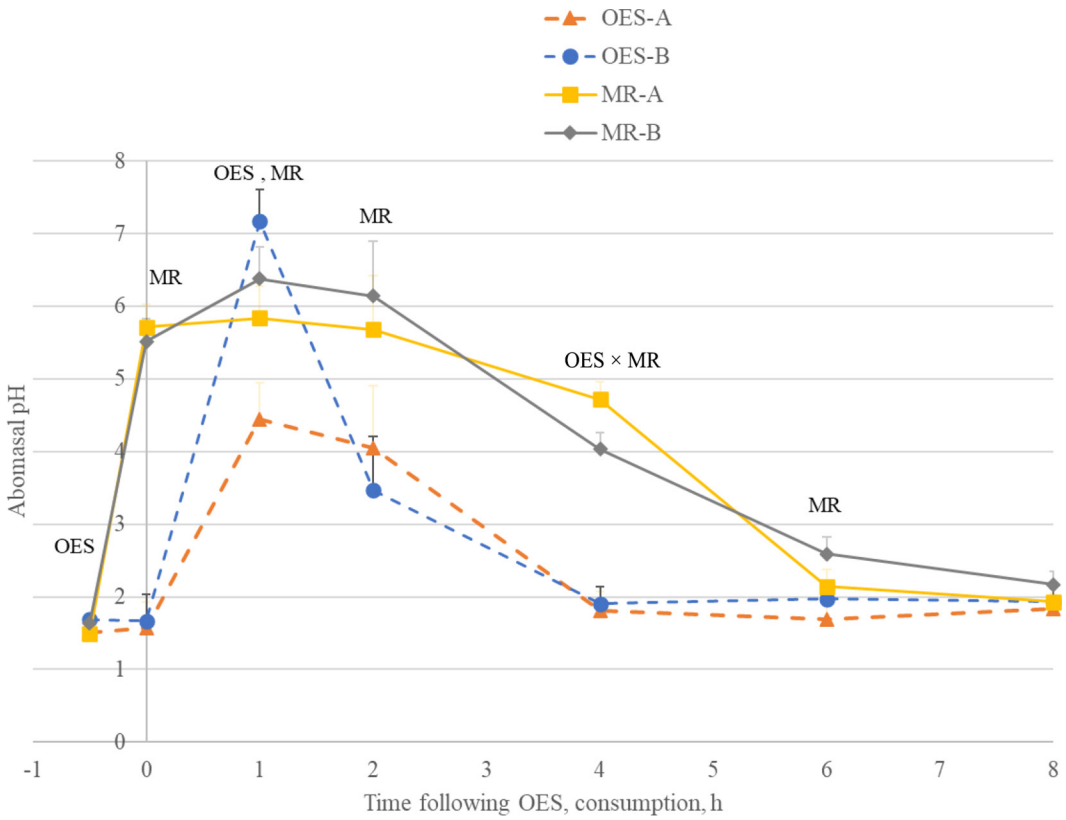

Figure 1. Abomasal pH in abomasal fluid samples was measured at −0.5, 0, 1, 2, 4, 6, and 8 h following consumption of 2 L of oral electrolyte solutions (OES). There was a tendency for an interaction between OES alkalinizing agent × fluid meal type × time ($P = 0.081$). Main effect and main effect interaction differences ($P \leq 0.05$) sliced by time are reported as OES for OES alkalinizing agent, milk replacer (MR) for fluid meal type, and OES × MR for an interaction between OES alkalinizing agent and fluid meal type. Data are presented as LSM ± SEM.

however, there was an OES alkalinizing agent × time effect ($P = 0.013$). Calves supplemented with bicarbonate OES had greater ex vivo *E. coli* counts in abomasal fluid relative to calves supplemented with acetate OES at 1 h after OES feeding. Further, there were MR × OES at 2 and 4 h after OES feeding, whereas calves fed either OES only, OES-A and OES-B, were not different from each other, but calves fed MR-B had greater ex vivo *E. coli* counts than calves fed MR-A at both time points.

Table 4. Summary of abomasal emptying indices in Holstein bull calves supplemented with oral electrolyte solutions (OES) containing different alkalinizing agents (sodium acetate, sodium bicarbonate) either with or without a prior milk replacer (MR) feeding; data are presented as mean ± largest SEM

Acetaminophen absorption[1]	Treatment[2]				Largest SEM	Fixed effects $P \leq$		
	OES-A	OES-B	MR-A	MR-B		OES	MR	OES × MR
C_{max} (ug/mL)	28.5[ab]	36.0[a]	23.4[bc]	17.7[c]	3.78	0.804	0.008	0.093
T_{max} (min)	100[a]	120[a]	300[b]	300[b]	54.4	0.848	0.004	0.848
AUC_{480}[3] (g × min/dL)	4,367	4,269	3,772	2,754	551.6	0.305	0.068	0.394

[a–c]Data values within a row that have differing superscript letters are significantly different ($P < 0.05$).

[1]Acetaminophen (50 mg/kg of BW) was blended into each OES to indirectly assess abomasal emptying rate; C_{max} represents the maximal plasma acetaminophen concentration observed in blood plasma; T_{max} represents the time point at which C_{max} occurred following consumption of the OES.

[2]Calves were randomly assigned to 1 of 4 dietary treatments that differed by the presence or lack of a milk meal (225 g of as-fed of 22% CP − 20% fat MR at 10.7% solids) administered 30 min before supplementation with 2 L of an OES containing 80 mmol/L of either sodium acetate or sodium bicarbonate as an alkalinizing agent.

[3]Area under the curve for 480 min (interval from feeding OES to 8 h after OES feeding).

Abomasum Fluid Bacterial Communities

Following quality filtering, denoizing, and removal of chimeric reads, a total of 544,390 high-quality reads (mean ± SD, 36,293 ± 23,387 per sample; n = 15) and 8,475 unique amplicon sequence variants (**ASV**) among 15 phyla, 105 families, and 355 genera were obtained from the samples taken intraoperatively during surgical cannulation on d 5. A total of 442,273 high-quality reads [mean ± SD, 31,591 ± 25,304 per sample; n = 14 (n = 3 for OES-A; n = 3 for OES-B; n = 4 for MR-A; n = 4 for MR-B)] and 6,528 ASV among 12 phyla, 86 families, and 265 genera were attained from the samples taken 6 h following OES consumption on d 9.

The top 10 abomasal bacterial communities comprised total relative abundances of greater than 99.9% for the intraoperative surgical cannulation samples acquired on d 5 (Figure 3A). *Bacteroidetes* (mean 34.7%), *Firmicutes* (mean 30.8%), and *Proteobacteria* (mean 18.4%) were the 3 most abundant phyla observed across all samples. Of the remaining phyla, only *Fusobacteria* (mean 8.4%), *Epsilonbacteraeota* (mean 4.0%), *Actinobacteria* (mean 3.2%), and *Verrucomicrobia* (0.29%) had mean relative abundance values greater than 0.10%.

On d 9 at 6 h following OES administration, fluid meal type, OES alkalinizing agent, or their interactions did not affect α diversity among treatments ($P \geq 0.123$). Beta diversity was also not affected by oral electrolyte alkalinizing agent type, presence of a MR feeding, or their interactions ($P > 0.05$). The top 10 abomasal bacterial communities comprised total relative abundances of greater than 99.9% for OE-A, OE-B, MR-A, and MR-B at the phylum level (Figure 3A). *Bacteroidetes* (mean 33.0%), *Firmicutes* (mean 26.3%), *Proteobacteria* (mean 20.4%), *Verrucomicrobia* (mean 10.9%), *Fusobacteria* (mean 4.1%), *Actinobacteria* (mean 2.7%), *Epsilonbacteraeota* (mean 2.3%), and *Patescibacteria* (mean 0.12%) were the most abundant phyla across all luminal fluid samples, representing mean relative abundance values greater than 0.10%. There was an OES alkalinizing agent × fluid meal type interaction for *Verrucomicrobia* ($P = 0.028$).

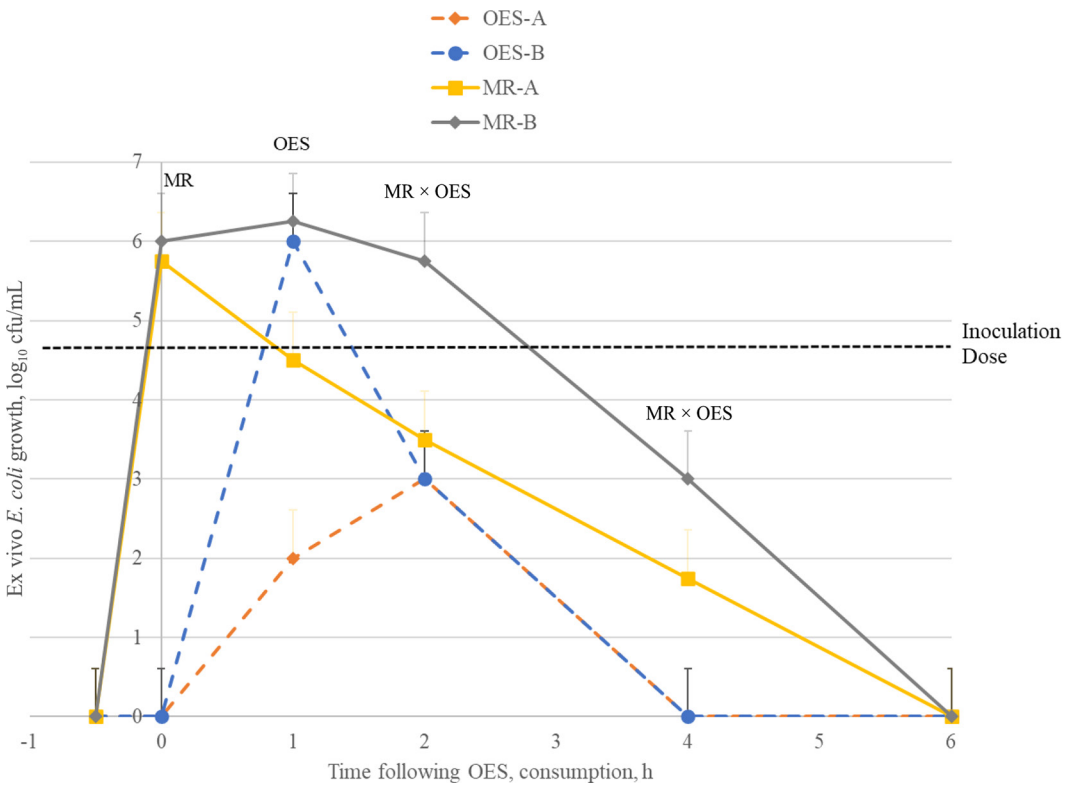

Figure 2. Ex vivo *Escherichia coli* growth in abomasal fluid samples at −0.5, 0, 1, 2, 4, and 6 h following oral electrolyte solutions (OES) supplementation. There was no interaction between OES alkalinizing agent × fluid meal type × time ($P = 0.206$). However, there was an OES alkalinizing agent × time interaction ($P = 0.013$). Main effect and main effect interaction differences ($P \leq 0.05$) sliced by time are reported as OES for OES alkalinizing agent, MR for fluid meal type, and OES × MR for an interaction between OES alkalinizing agent and fluid meal type. OES-A and OES-B treatment groups received only 2 L of OES containing either 80 mmol/L of acetate or bicarbonate, respectively; MR-A and MR-B treatment groups received a milk replacer (MR) feeding 30 minutes prior to being fed the OES containing either acetate or bicarbonate, respectively. Data are presented as LSM ± SEM.

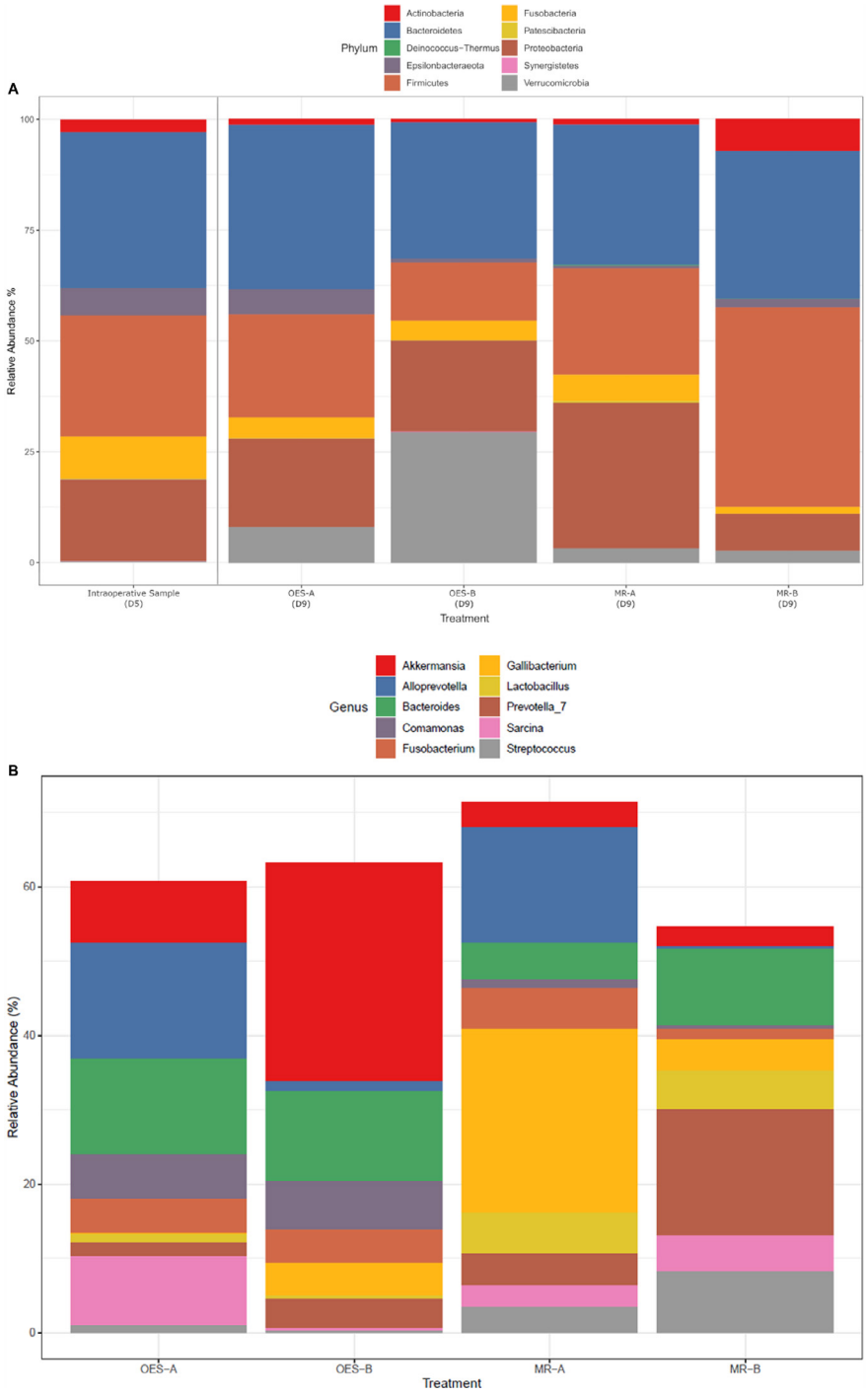

Figure 3. Bacterial communities in the abomasum. (A) The top 10 most abundant bacterial communities together comprised a total relative abundance of >99.9% for both the intraoperative surgical cannulation samples acquired on d 5 and for the different treatments collected 6 h after their respective treatments on d 9. *Bacteroidetes* (mean 33.0%), *Firmicutes* (mean 26.3%), and *Proteobacteria* (mean 20.4%) were the most abundant phyla across all luminal fluid samples after oral electrolyte treatments; and (B) the top 10 most abundant genera covered a total relative abundance of 60.7, 63.3, 71.3, and 54.6% for OES-A, OES-B, MR-A, and MR-B, respectively. *Akkermansia* (mean 10.9%), and *Streptococcus* (mean 8.7%), and *Lactobacillus* (mean 7.7%) were the most abundant genera. There were oral electrolyte alkalinizing agent type × presence of previous milk replacer (MR) feeding interactions for *Akkermansia* ($P = 0.028$) and *Lachnoclostridium* ($P = 0.024$). Calves administered MR and oral electrolyte solutions (OES) had greater relative abundances of *Lactobacillus* and *Streptococcus* ($P \leq 0.037$); however, *Bifidobacterium* was increased in calves supplemented with OES only ($P = 0.040$). Calves supplemented with a bicarbonate-based OES tended to have a greater relative abundance of *Escherichia* and *Shigella* ($P = 0.054$) compared with those supplemented with an acetate-based OES. OES-A and OES-B treatment groups received only 2 L of OES containing either 80 mmol/L of acetate or bicarbonate, respectively; MR-A and MR-B treatment groups received a milk replacer (MR) feeding 30 minutes prior to being fed the OES containing either acetate or bicarbonate, respectively.

The top 10 abomasal bacterial communities at the genus level covered total relative abundances of 60.7, 63.3, 71.3, and 54.6% for OE-A, OE-B, MR-A, and MR-B, respectively (Figure 3B). *Akkermansia* (mean 10.9%), and *Streptococcus* (mean 8.7%), and *Lactobacillus* (mean 7.7%) were the most abundant genera across all d 9 abomasal luminal fluid samples. There were OES alkalinizing agent × fluid meal type interactions for *Akkermansia* ($P = 0.028$) and *Lachnoclostridium* ($P = 0.024$). A fluid meal type effect was observed for relative abundances of *Lactobacillus* and *Streptococcus* ($P \leq 0.037$), with greater amounts of these bacterial genera observed in calves administered MR and OES. However, *Bifidobacterium* was increased in calves supplemented with OES only ($P = 0.040$). A tendency for an OES alkalinizing agent effect was present for *Escherichia* and *Shigella* ($P = 0.054$); calves fed a bicarbonate-based OES had a greater relative abundance of these bacterial populations compared with calves fed an acetate-based OES.

DISCUSSION

Supplementation of OES represents a gold standard in the rehydration treatment process for scouring and dehydrated calves. The composition of an OES and the timing of OES administration for optimizing clinical outcomes remains unknown. Because the energetic density of commercial OES is usually not sufficient to meet daily requirements for maintenance coupled with severe bodily fluid losses, it is recommended that scouring calves receive their daily milk allowance in addition to OES (Smith, 2009). Traditionally, OES were recommended to be offered in-between milk feedings, usually 4 to 6 h after the morning or evening milk feeding. However, calf-raising operations experience logistical and labor limitations thus OES administration may occur in closer proximity with milk feeding. This study investigated the effects of oral electrolyte alkalinizing agent and fluid meal type on the environment of the abomasum in neonatal Holstein calves. Data from this experiment indicate that feeding a MR meal 30 min before administering an OES results in a sustained increase in postprandial abomasal pH and delayed AER. Together, these result in increased ex vivo counts of *E. coli* bacteria. Further, using sodium bicarbonate as the alkalinizing agent in MR-B or OES-B treated calves reduced abomasal acidity and permitted an increased ex vivo *E. coli* counts compared with calves fed MR-A or OES-A.

The type of alkalinizing agents used in an OES to correct the metabolic acidosis common with dehydration may temporarily disrupt the endogenous acidity of the abomasal compartment. Consistent with previous data, sodium bicarbonate efficiently alkalinized the abomasum to a greater extent than using sodium acetate as the alkalinizing agent by directly binding to luminal hydronium ions to form carbon dioxide and water (Marshall et al., 2008; Sen et al., 2009). The OES-B calves achieved a greater mean maximal postprandial pH compared with OES-A calves. Sodium bicarbonate exerts its alkalinizing effects immediately within the lumen of abomasum, whereas other alkalinizing agents such as acetate and propionate rely on hepatic metabolism before attaining their alkalinizing ability. The rates of systemic alkalinization of both acetate and propionate appear comparable to sodium bicarbonate (Sen et al., 2009). Sustained elevations in postprandial abomasal pH compromises the natural acidic barrier that provides antimicrobial activity. Similar clinical outcomes related to the treatment of systemic acidemia using either an acetate or propionate-based OES may be achieved without a prolonged compromise to the endogenous acidity of the abomasum.

Acetaminophen absorption pharmacokinetics were validated as an indirect measurement of the fluid phase of gastric emptying in preweaning calves. Marshall et al. (2005) determined that T_{max} provided the most accurate index of AER using acetaminophen absorption × time data in calves. In instances where total volume administered between dietary treatments is unequal, C_{max} may be imprecise given that the amount of acetaminophen per unit volume differs when using a standardized dosage per animal (50 mg/kg BW). The T_{max} alleviates this problem because it occurs independent of the C_{max} value. Our data further support this, whereas the T_{max} of the calves fed MR and OES was delayed when compared with the calves fed OES only, but C_{max} and AUC were not different among calves fed MR and OES versus OES only.

Total volume of an ingested meal prolongs the duration of time fluid remains in a calf's abomasum. On average, calves fed 2 L of a 20% CP and 20% fat MR empty their abomasum in approximately 190 to 206 min via maximal acetaminophen absorption indices (Marshall et al., 2005). Calves in the MR-A and MR-B treatment groups received both MR and OES at a total overall volume of 4.10 L (e.g., 2.10 L of MR + 2.0 L of OES). Using T_{max} as an index of AER, there was a presence of a previous MR meal main effect. Calves offered both MR and OES took 300 min to empty their abomasum compared with 100 and 120 min for OES-A and OES-B calves, respectively. The prolonged AER in MR and OES fed calves was similar to Holstein calves fed a higher plane of nutrition, 1,100 g of a 26% protein and 20% fat fed at 15.5% solids divided between 2

feedings (Kasl, 2020). Additionally, the caloric content of the liquid meal affects AER, whereas solutions of greater caloric density have slower AER to supply a more constant supply of those nutrients to the intestines. Marshall et al. (2005) reported that calves fed 2 L of a 20% protein and 20% fat MR had reduced AER when compared with calves fed similar volumes of various isotonic OES solutions. Therefore, total fluid meal volume or caloric density, not OES alkalinizing agent, affected AER when both MR and OES are administered within a shortened feeding interval. It is worth noting that both of these OES were only slightly hypertonic, whereas Sen et al. (2006) reported reduced abomasal emptying of hypertonic solutions of either sodium bicarbonate or glucose relative to their isotonic solutions, respectively.

Abomasal pH and the presence of nutrients may influence the ability of potentially pathogenic bacteria to grow in the abomasum as hypothesized by Smith (2009) in his review article. Feeding MR and OES or feeding OES-B increased ex vivo *E. coli* growth potential. Enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and *Salmonella* spp. are common gastroenteric etiologic agents responsible for scouring events in calves less than 3 wk of age. The acidic environment of the abomasum has a bactericidal effect on these bacteria when pH is less than 2.5 to 3.0. Increasing the alkalinity of the gastric compartment above a pH of 5.0 may permit the replication of coliforms and *Salmonella* spp. White et al. (1998) experimentally induced *E. coli* K99 diarrhea in 1- to 2-wk-old Holstein calves by drenching each animal with a solution containing 4 g of sodium bicarbonate before the oral challenge. The 8 h postprandial gastric pH was greater for calves offered both MR and OES exceeding thresholds for endogenous bactericidal control of pathogenic bacteria. Administering both MR and OES results in greater postprandial gastric pH values, prolonged rates of abomasal emptying, and increased ex vivo *E. coli* luminal fluid growth that temporarily compromise abomasal health and environmental homeostasis in exchange for aggressive rehydration and correction of systemic acidemia. Young calves with spontaneous diarrhea have increased concentrations of *E. coli* in the intestines with the distal jejunum and ileum increasing to the greatest degree (Smith 1962; Youanes and Herdt, 1987). Therefore, any dietary strategies that may promote the growth of gram-negative bacteria, such as *E. coli*, may further exacerbate the overgrowth of these bacteria in the distal small intestines. Future research should investigate the role of different OES therapy on the microbial communities in the distal small intestines and whether feeding an OES with bicarbonate exacerbates diarrhea more than an OES with acetate.

Further complicating the pathogenesis of diarrhea in calves, Kirchner et al. (2015) demonstrated using ultrasonography that diarrheic calves have delayed abomasal emptying compared with healthy calves when feeding a blended solution of whole milk or MR and a bicarbonate-based OES. Evidence of prolonged abomasal passage rates in scouring calves were further supported by acetaminophen absorption testing data in diarrheic calves less than 2 wk of age fed 75 g of a bicarbonate-based OES in 2 L of a 20% CP and 17% fat MR. Median time to maximal acetaminophen concentration was 300 min in healthy calves fed MR and OES compared with 390 min for calves with diarrhea. Blending MR and OES together appeared to have an additive effect on delaying AER, which is likely associated with the increased osmolality (Hildebrandt et al., 2020). Apparently healthy calves appear to have a greater physiologic capability to handle the increased volume associated with feeding MR and OES meals within a shortened time interval compared with those with concurrent gastrointestinal disease. Administering OES containing sodium acetate decreases the ex vivo ability of *E. coli* to grow in abomasal luminal fluid compared with calves supplemented with OES containing sodium bicarbonate. Further supporting the benefit of using an OES containing sodium acetate over sodium bicarbonate was the tendency for a reduction in the presence of *Escherichia* and *Shigella* in the abomasum of calves 6 h after feeding a single feeding of OES.

The majority of the published literature on the gastrointestinal bacterial communities of preweaning calves has prioritized the reticuloruminal compartment and intestinal tract (Dias et al., 2018). Added emphases have been placed on identifying shifts in the microbiome in relation to improvements in performance and the weaning period (Meale et al., 2017). Bacteroidetes had the greatest phylum relative abundance within the intraoperative abomasal fluid samples. This is consistent with Yeoman et al. (2018), who observed *Bacteroidetes* as having the greatest relative abundance in the abomasum of healthy calves during the first 3 weeks of age. Similarly, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* represented the phyla with the greatest relative abundances at 6 h postprandially on d 9. Alpha diversity measurements are more likely to differ by gastrointestinal region rather than by developmental stage (Dias et al., 2018). Although α diversity measurements did not differ among treatments, it is unknown whether or not shifts in the gastrointestinal bacterial communities occurred in regions of the gastrointesintal tract distal to the abomasum. Fibrolytic bacterial populations, including *Fibrobacter* and *Ruminococcus* were absent from the top 10 bacterial genera at d 9 despite ad libitum access to calf starter. Guzman et al.

(2016) observed an increase in abomasal *Ruminococcus flavefaciens* abundance at 14 d of age, but not at 7 d of age, in calves administered both milk and calf starter. Likely contributing to the undetectable abundance of fibrolytic bacteria across treatments at d 9 was a lack of consistent, appreciable intake of solid feed.

CONCLUSIONS

Preweaning calves are highly susceptible to dehydration given their susceptibility to scouring gastrointestinal diseases. Oral electrolyte solutions represent an effective, economical on-farm therapy to maintain or restore hydration and correct metabolic acidemia. Sodium bicarbonate reacts within luminal fluid resulting in abomasal alkalinization whereas acetate requires hepatic metabolism to acquire its alkalinizing ability. Administering MR and OES within a short feeding interval results in sustained elevations in abomasal pH, delayed abomasal emptying, and increased ex vivo growth of potentially pathogenic bacteria. Future research should investigate if it is preferable to feed an OES before MR, because the OES only treatments had rapid abomasal emptying and therefore, much of the oral electrolyte could leave the abomasum before the MR feeding. Regardless, the bicarbonate-based OES increases the potential for luminal coliform growth, likely partially due to the magnitude of the increase in abomasal pH. Utilizing sodium acetate as an alkalinizing agent when supplementing OES soon after a milk meal may reduce the opportunity for *E. coli* growth within abomasal luminal fluid. A small sample size was used in this study due to the nature of the data collected; future research should investigate if feeding OES with a bicarbonate buffer exacerbates diarrhea more than feeding an OES with an acetate buffer.

ACKNOWLEDGMENTS

The authors thank Courtney Hoff, Iuli Guimaraes, Thiago Silva, Paulo Menta, Maria Luiza Celestino, and Leticia Fernandes in the Department of Veterinary Sciences at Texas Tech University for their assistance with abomasum cannulation and sample processing throughout the experiment. The authors thank the staff at the USDA-ARS in Lubbock, Texas, including Jeff Carroll, Rand Broadway, Nicole Sanchez, and Jeff Dailey, for the research facility and laboratory materials used to complete this study. M.A. Ballou has equity ownership in MB Nutritional Sciences LLC, which donated the MR used in the study and formulated the oral electrolytes fed in the study. No other authors declared any conflicts of interest.

REFERENCES

Ahmed, A. F., P. D. Constable, M. McCallister, and N. Misk. 2005. Abomasal cannulation in the milk-fed calf using a 7 mm polyurethane tube. J. Vet. Med. A Physiol. Pathol. Clin. Med. 52:39–42. https://doi.org/10.1111/j.1439-0442.2004.00686.x.

Burgstaller, J., T. Wittek, and G. W. Smith. 2017. Invited review: Abomasal emptying in calves and its potential influence on gastrointestinal disease. J. Dairy Sci. 100:17–35. https://doi.org/10.3168/jds.2016-10949.

Callahan, B. J., P. J. McMurdie, and S. P. Holmes. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11:2639–2643. https://doi.org/10.1038/ismej.2017.119.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016a. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13:581–583. https://doi.org/10.1038/nmeth.3869.

Callahan, B. J., K. Sankaran, J. A. Fukuyama, P. J. McMurdie, and S. P. Holmes. 2016b. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses. F1000Res. 5:1492.

Chen, Y., Y.-H. Tsai, B.-J. Tseng, and S.-H. Tseng. 2019. Influence of growth hormone and glutamine on intestinal stem cells: A narrative review. Nutrients 11:1941. https://doi.org/10.3390/nu11081941.

Dias, J., M. I. Marcondes, S. Motta de Souza, B. M. E. Cardoso da Mata e Silva, M. Fontes Noronha, R. Tassinari Resende, F. S. Machado, H. Cuquetto Mantovani, K. A. Dill-McFarland, and G. Suen. 2018. Bacterial community dynamics across the gastrointestinal tracts of dairy calves during preweaning development. Appl. Environ. Microbiol. 84:e02675-17. https://doi.org/10.1128/AEM.02675-17.

Edgar, R. C., and H. Flyvbjerg. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31:3476–3482. https://doi.org/10.1093/bioinformatics/btv401.

Glassman, S. I., and J. B. Martiny. 2018. Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. MSphere 3:e00148-18. https://doi.org/10.1128/mSphere.00148-18.

Guzman, C. E., L. T. Bereza-Malcolm, B. De Groef, and A. E. Franks. 2016. Uptake of milk with and without solid feed during the monogastric phase: Effect on fibrolytic and methanogenic microorganisms in the gastrointestinal tract of calves. Anim. Sci. J. 87:378–388. https://doi.org/10.1111/asj.12429.

Hildebrandt, T., E. Scheuch, W. Weitschies, F. Schneider, M. Grimm, L. Bachmann, and I. Vervuert. 2020. Abomasal emptying rate of diarrhoeic and healthy suckling calves fed with oral rehydration solutions. J. Anim. Physiol. Anim. Nutr. (Berl.) 104:462–469. https://doi.org/10.1111/jpn.13306.

Kasl, B. K. 2020. The effects of nutritional and feeding management factors on the abomasal environment in preweaned dairy calves. Ph.D. Dissertation. Texas Tech University, Lubbock.

Kirchner, D., L. Schwedhelm, J. Wenge, I. Steinhöfel, C. Heinrich, M. Coenen, and L. Bachmann. 2015. Ultrasonographic imaging of abomasal milk clotting and abomasal diameter in healthy and diarrheic calves. Anim. Sci. J. 86:929–936. https://doi.org/10.1111/asj.12382.

MacPherson, J. A. R., H. Berends, L. N. Leal, J. P. Cant, J. Martín-Tereso, and M. A. Steele. 2016. Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. J. Dairy Sci. 99:8007–8017. https://doi.org/10.3168/jds.2015-10826.

Marshall, T. S., P. D. Constable, S. S. Crochik, and T. Wittek. 2005. Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption. Am. J. Vet. Res. 66:364–374. https://doi.org/10.2460/ajvr.2005.66.364.

Marshall, T. S., P. D. Constable, S. S. Crochik, T. Wittek, D. E. Freeman, and D. E. Morin. 2008. Effect of suckling an isotonic solution of sodium acetate, sodium bicarbonate, or sodium chloride on ab-

omasal emptying rate and luminal pH in calves. Am. J. Vet. Res. 69:824–831. https://doi.org/10.2460/ajvr.69.6.824.
McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217.
Meale, S. J., F. Chaucheyras-Durand, H. Berends, L. L. Guan, and M. A. Steele. 2017. From pre-to postweaning: Transformation of the young calf's gastrointestinal tract. J. Dairy Sci. 100:5984–5995. https://doi.org/10.3168/jds.2016-12474.
Nouri, M., and P. D. Constable. 2006. Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. J. Vet. Intern. Med. 20:620–626. https://doi.org/10.1111/j.1939-1676.2006.tb02906.x.
Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., O'hara, R., Simpson, G., Solymos, P., Stevens, M., & Wagner, H. (2013). Community ecology package. R package version, 2.0-2. https://cran.r-project.org/web/packages/vegan/index.html.
Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219.
Schwedhelm, L., D. Kirchner, B. Klaus, and L. Bachmann. 2013. Experimentally induced hyperchloremic and DL-lactic acidosis in calves: An attempt to study the effects of oral rehydration on acid-base status. J. Dairy Sci. 96:2464–2475. https://doi.org/10.3168/jds.2012-6077.
Sen, I., V. Altunok, M. Ok, A. Coskun, and P. D. Constable. 2009. Efficacy of oral rehydration therapy solutions containing sodium bicarbonate or sodium acetate for treatment of calves with naturally acquired diarrhea, moderate dehydration, and strong ion acidosis. J. Am. Vet. Med. Assoc. 234:926–934. https://doi.org/10.2460/javma.234.7.926.
Sen, I., P. D. Constable, and T. S. Marshall. 2006. Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rates in calves. Am. J. Vet. Res. 67:1377–1384. https://doi.org/10.2460/ajvr.67.8.1377.
Smith, G. W. 2009. Treatment of calf diarrhea: Oral fluid therapy. Vet. Clin. North Am. Food Anim. Pract. 25:55–72. https://doi.org/10.1016/j.cvfa.2008.10.006.
Smith, G. W., A. F. Ahmed, and P. D. Constable. 2012. Effect of orally administered electrolyte solution formulation on abomasal luminal pH and emptying rate in dairy calves. J. Am. Vet. Med. Assoc. 241:1075–1082. https://doi.org/10.2460/javma.241.8.1075.
Smith, G. W., and J. Berchtold. 2014. Fluid therapy in calves. Vet. Clin. North Am. Food Anim. Pract. 30:409–427.
Smith, H. W. 1962. Observations on the etiology of neonatal diarrhoea (scours) in calves. J. Pathol. Bacteriol. 84:147–168. https://doi.org/10.1002/path.1700840117.
Urie, N. J., J. E. Lombard, C. B. Shivley, C. A. Kopral, A. E. Adams, T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244. https://doi.org/10.3168/jds.2017-14019.
Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261–5267. https://doi.org/10.1128/AEM.00062-07.
Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569–577. https://doi.org/10.1111/j.1939-1676.2000.tb02278.x.
White, D. G., C. K. Johnson, and V. Cracknell. 1998. Comparison of danofloxacin with baquiloprim/sulphadimidine for the treatment of experimentally induced *Escherichia coli* diarrhoea in calves. Vet. Rec. 143:273–276. https://doi.org/10.1136/vr.143.10.273.
Wickramasinghe, H. K. J. P., A. J. Kramer, and J. A. D. R. N. Appuhamy. 2019. Drinking water intake of newborn dairy calves and its effects on feed intake, growth performance, health status, and nutrient digestibility. J. Dairy Sci. 102:377–387. https://doi.org/10.3168/jds.2018-15579.
Yeoman, C. J., S. L. Ishaq, E. Bichi, S. K. Olivo, J. Lowe, and B. M. Aldridge. 2018. Biogeographical differences in the influence of maternal microbial sources on the early successional development of the bovine neonatal gastrointestinal tract. Sci. Rep. 8:3197. https://doi.org/10.1038/s41598-018-21440-8.
Youanes, Y. D., and T. H. Herdt. 1987. Changes in the small intestinal morphology and flora associated with decreased energy digestibility in calves with naturally occurring diarrhea. Am. J. Vet. Res. 48:719–725.

ORCIDS

B. A. Kasl https://orcid.org/0000-0002-9762-5335
V. S. Machado https://orcid.org/0000-0003-1513-8216
M. T. Henniger https://orcid.org/0000-0001-7739-8932
P. R. Myer https://orcid.org/0000-0002-1980-2105
M. A. Ballou https://orcid.org/0000-0002-9843-1196