Investigation of ammonium lactate supplementation on fermentation end products and bacterial assimilation of nitrogen in dual-flow continuous culture

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ABSTRACT

Supplements investigated throughout the present study are produced by fermenting lactose that is present in whey to lactate, yielding products differing in ammonium relative to lactate concentrations and in physical form (liquid or dry). Trials 1 and 2 investigated Lacto-Whey (LW; Fermented Nutrition Corp., Luxemburg, WI) and GlucoBoost (GB; Fermented Nutrition Corp.), respectively, using dual-flow continuous culture systems ($n = 4$), each with a 4×4 Latin square design. A greater proportion of nonprotein nitrogen was present in GB than in LW. In trial 1, the treatment with LW was isonitrogenously dosed against soybean meal (SBM) as a control (no LW) and factorialized with either a wheat- or corn-based concentrate (55% inclusion rate, dry matter basis). We hypothesized that LW would increase propionate production and that the combination of +LW with wheat would increase bacterial assimilation of $\text{NH}_3\text{-N}$ into cellular N. No differences were observed for total volatile fatty acid (VFA) production per day. However, treatment $\times$ time interactions revealed that +LW increased lactate concentration at 0, 0.5, and 1 h and tended to increase molar percentage of propionate at 1 and 1.5 h postfeeding, documenting the immediate availability of lactate converted to propionate in the +LW treatments. The main effect of corn increased the proportion of bacterial N derived from $\text{NH}_3\text{-N}$. Trial 2 was designed to investigate GB; isonitrogenous treatments included an SBM control, crystal GB, liquid GB (LGB), and LGB with yeast culture, which were dosed twice daily. We hypothesized that GB would increase propionate production and bacterial assimilation of $\text{NH}_3\text{-N}$; the

combination of LGB and yeast culture was expected to have a positive additive effect, yielding the greatest VFA production and bacterial $\text{NH}_3\text{-N}$ assimilation. No differences were observed for total VFA production; however, LGB decreased molar percentage of acetate and increased propionate and butyrate molar percentages. There were no differences in non- $\text{NH}_3\text{-N}$ flow or microbial N flow. Under the conditions of our studies, lactate in LW and GB was fermented extensively to propionate, and microbial protein synthesis in these treatments was comparable with that in SBM controls. **Key words:** ammonium lactate, rumen fermentation, microbial protein synthesis, continuous culture

INTRODUCTION

With increases expected in the global demand for traditional grains, use of by-products will become increasingly important as a way to decrease feed costs within US animal production systems (Johansson, 2015). Some producers include whey in their dairy rations for added energy and protein; however, supplementing sugars, such as lactose in whey, has yielded inconsistent results, specifically with respect to microbial protein synthesis (Oba, 2011) and animal efficiency of N utilization (Broderick et al., 2008; Penner and Oba, 2009). Compared with feeding raw whey products, fermenting lactose to lactate might help circumvent potential complications by improving stability during storage and lowering transportation costs of a drier product. Moreover, the nutritional quality might be improved because of decreased variation in nutrient concentrations and a more consistently increased yield of gluconeogenic precursors absorbed by the dairy cow.

Lactate is a key intermediate in ruminal fermentation and is metabolized via different pathways, resulting in the production of different VFA. Some strains of *Megasphaera elsdenii* will preferentially utilize lactate over glucose (Weimer and Moen, 2013), producing either propionate via the acrylate pathway or acetate and butyrate via pyruvate. In addition, *Selenomonas*

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ruminantium ssp. *lactilytica* can also utilize lactate, producing acetate, propionate, and succinate (Nagaraja and Titgemeyer, 2007). Protozoa, most notably *Entodinium* spp., also metabolize lactate primarily to butyrate (Brossard et al., 2004). Greater ruminal lactate concentrations in defaunated (protozoa removed) animals suggest that protozoa play a key role in lactate metabolism (Newbold et al., 2015). Increased lactate availability and subsequent utilization by microbes should facilitate cellular carbon conversion into bacterial products and increase the assimilation of $\text{NH}_3\text{-N}$ into bacterial AA and nucleic acids. For this reason, supplementing lactate and ammonium together has the potential for a positive additive effect.

Probiotics offer potential to modulate lactate accumulation, perhaps explaining why supplemental yeast culture (YC) sometimes increases total VFA concentration (Desnoyers et al., 2009). Yeast culture might increase abundance of lactate-utilizing bacteria, such as *M. elsdenii* (Newbold et al., 1996; Callaway and Martin, 1997) and *S. ruminantium* (Martin, 1998), which should increase conversion of lactate to propionate (the key gluconeogenic precursor) or to butyrate (a key fuel and metabolite for the rumen epithelium; Oba, 2011).

We aimed to evaluate the effects of ammonium lactate-based supplements on VFA production and bacterial assimilation of $\text{NH}_3\text{-N}$. Trial 1 investigated Lacto-Whey (LW; Fermented Nutrition Corp., Luxemburg, WI) from the standpoint of conversion of lactate to VFA with contrasting grain sources (corn or wheat). Lactate is neutralized with NH_3 to maintain a pH of about 5.5 to maximize lactate yield (37% lactate on an as-is basis), with the CP containing about 75 to 77% $\text{NH}_3\text{-N}$, 17% whey protein, and 6 to 8% spent *Lactobacillus bulgaricus* cells (Reddy et al., 1976). Although LW was researched decades ago as a substitute for soybean meal in dairy rations, presumably through assimilation into rumen microbial protein (Huber et al., 1976), no direct comparison has been made for microbial N measurements. Renewed interest in fermented whey products has justified the need to study assimilation of LS $\text{NH}_3\text{-N}$ into microbial N. We hypothesized that LW in combination with the wheat-based diet would increase propionate production while also increasing bacterial assimilation of $\text{NH}_3\text{-N}$. Next-generation sequencing was performed to evaluate a potential shift in the bacterial and archaeal communities.

Trial 2 evaluated the effects of feeding GlucoBoost (GB; Fermented Nutrition Corp.) as a crystal (CGB) or liquid (LGB) without and with YC compared with soybean meal (SBM). The LGB is similar to LW but with slightly less $\text{NH}_3\text{-N}$, whereas the CGB uses Ca in whey to crystallize the ammonium lactate (Marri-

ott, 1985). Because *Lactobacillus bulgaricus* produces L- and potentially considerable (up to 90%) D-lactate (Bernard et al., 1991), we surmised that addition of YC might increase conversion of total lactate, particularly D-lactate, primarily to propionate (via succinate), as did an extract of *Aspergillus oryzae* (Nisbet and Martin, 1993). We hypothesized that GB inclusion would increase propionate production; the combination of liquid GB and YC was expected to have a positive additive effect and yield the greatest VFA production and bacterial $\text{NH}_3\text{-N}$ assimilation. The combination of both studies provides an opportunity to evaluate lactate metabolism in continuous cultures, which allows direct quantification of net VFA production while maintaining both bacterial and protozoal lactate-metabolizing communities.

MATERIALS AND METHODS

Experimental Design

Trial 1. Rumen fluid was collected and pooled from 2 ruminally cannulated Jersey cows housed at The Ohio State University's Waterman Dairy Center to be used as inocula. Donor cows were fed a typical lactating diet that contained corn silage (58.1%), grain mix (23.2%), alfalfa (14.2%), and whole cottonseed (4.3%); the remaining amount represented mineral supplements to meet or slightly exceed nutrient requirements. All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee. Collected material was immediately squeezed through 2 layers of cheesecloth into prewarmed beverage coolers and maintained at 39°C during transportation back to the laboratory. In less than 40 min from sampling, the inoculum was delivered with an equal part of buffer (totaling half of the 1.71 ± 0.25 L of working volume) into each of the 4 individual fermentation systems that featured a continuous influx of buffer solution, a heating element to maintain the culture at 39°C, and an impeller (50 rpm) to maintain adequate mixing. The buffer solution was made according to Weller and Pilgrim (1974) except that it excluded urea to maintain (and not exceed) desired $\text{NH}_3\text{-N}$ concentrations (5–10 mg/dL, approximately 4 h postfeeding). Total buffer input and liquid passage rate were maintained at 7%/h; passage rate of solids was 5%/h. Modified filters (50- μm pore size) were used on filtrate pumps to retain protozoa (Karnati et al., 2009).

Fermentors were administered a pelleted diet (60 g of DM/d; 55:45 concentrate:forage diet; Table 1) and their respective treatments (10 mL of slurry) twice daily at 0800 and 2000 h. Diets contained either a

wheat- or corn-based concentrate and were supplemented with either SBM or LW in a 2×2 factorial treatment arrangement in a 4×4 Latin square design. Both SBM and LW provided 1.15 g/d of CP (as assessed by Kjeldahl; AOAC, 1990). The LW was scaled from a feeding rate of 1.14 kg (as-fed basis) per 22.7 kg/d of DMI. It was projected to provide 12.3 mmol/d of lactate, with lactate in LW analyzed using HPLC (AOAC, 1990; method 986.13) by Midwest Laboratories (Omaha, NE). To facilitate sinking and avoid immediate outflow during treatment administration, SBM was ground with a mortar and pestle and wetted with distilled water before being poured into the fermentor. After 2 d of adapting microbes and 5 d of dietary adaptation, sampling was from d 7 to 10. Beginning on d 2, all continuous culture systems were monitored for CH_4 and dihydrogen (H_2) present in the headspace via

a Micro-Oxymax respirometer (Columbus Instruments Inc., Columbus, OH), the details of which are described by Wenner et al. (2017). Ammonium sulfate (10% atom percentage excess ^{15}N ; Isotec, Miamisburg, OH) was infused via the buffer starting on d 5 at an average rate of 0.071 g/d.

Trial 2. Rumen fluid collection, fermentor operation, buffer solution preparation, and ^{15}N -labeled ammonium sulfate infusion were completed in the same way as described in trial 1. The total working volume of the fermentor systems was 1.70 L (range: ± 0.20 L). Fermentors were administered a pelleted diet (61.8 g of DM/d; 50:50 concentrate:forage diet; Table 1) and their respective treatments twice daily at 0800 and 2000 h. The 4 treatments included an SBM control (CON), CGB, LGB, and LGB with YC (LGB+YC). The CGB, LGB, and LGB+YC treatments contained

Table 1. Ingredient and nutrient composition of base diets for trials 1 and 2 administered to continuous cultures

Item	Trial 1 ¹		Trial 2 ²
	Corn	Wheat	Base diet
Diet ingredient, ³ %			
Alfalfa meal	50.0	50.0	48.8
Corn grain	32.0	—	33.1
Wheat	—	36.6	—
Soybean hulls	15.6	11.4	7.98
Corn distillers grains with solubles	—	—	8.21
Vegetable oil	0.90	0.90	0.43
Dicalcium phosphate	0.60	0.60	—
Trace-mineralized salt ⁴	0.45	0.45	0.82
Soybean meal, 48% CP	—	—	0.51
Urea	0.40	—	—
Magnesium oxide	0.10	0.10	0.10
Selenium 200	—	—	0.10
Diet composition, %			
CP ⁵	15.7	15.7	15.0
NDF	36.5	36.6	33.6
NSC ⁶	28.5	32.4	—
Starch	—	—	24.1

¹In trial 1, continuous cultures received 60.0 g/d (DM basis) of either a corn- or wheat-based concentrate to which was added a slurry without (–LW) or with (+LW) Lacto-Whey (Fermented Nutrition Corp., Luxemburg, WI) twice daily. Lacto-Whey contains 42.0% ammonium lactate (37.0% lactate) on an as-is basis (analyzed to contain 47.2% DM and projected to provide 12.3 mmol/d of lactate split into 2 doses/d; 6.16 mmol/dose).

²In trial 2, continuous cultures received a base diet of 60.0 g/d (DM basis) to which was added a slurry with a control (CON) or another ammonium lactate product, GlucoBoost (GB; Fermented Nutrition Corp.), in 1 of 3 forms: crystal (CGB), liquid (LGB), or LGB plus yeast culture (LGB+YC). Treatments were projected to provide 7.80, 14.12, and 13.92 mmol/d of lactate (3.90, 7.06, and 6.96 mmol of lactate/dose), respectively.

³Composition of base diet excluding added soybean meal, LW, LGB, CGB, or LGB+YC.

⁴Contained Na (36.6%), Cl (56.4%), Zn (3,500 mg/kg), Mn (2,800 mg/kg), Fe (1,750 mg/kg), Cu (350 mg/kg), I (70 mg/kg), and Co (70 mg/kg).

⁵Total CP provided from each diet. Soybean meal (53% CP), LW, CGB, LGB, and LGB+YC contained 50.7, 80.6, 47.1, 58.0, and 56.9% CP on a DM basis, respectively. Ammonium lactate treatments were administered along with appropriate amounts of additional soybean meal to make all treatments isonitrogenous. In trial 1, LW provided 11.8% of total CP, and total CP of the base diets plus dosed treatments was 9.73 g/d in all diets. In trial 2, CGB, LGB, and LGB+YC provided 3.5, 8.5, and 7.2% of total CP, respectively, and total CP of the base diets plus dosed treatments was 10.1 g/d in all diets.

⁶Included starch and sugars.

47.6, 71.6, and 64.9% CP on a DM basis, respectively. All treatments were made isonitrogenous by including appropriate amounts of SBM with the treatment dose, which was prepared as in trial 1. The CGB, LGB, and LGB+YC had 78.0, 53.1, and 52.3% lactate, respectively. Each product was dosed in different amounts based on actual field dosage rates (0.34 kg as fed of CGB/22.7 kg of DMI and 0.91 kg as fed of LGB or LGB+YC/22.7 kg of DMI) as scaled to fermentor intakes. The CGB forms a crystal with 1 mol of $\text{Ca}^{++}/2$ mol of lactate (Marriott, 1985), so its inclusion rate in rations is limited by its Ca concentration. Therefore, the amount of lactate provided by CGB and LGB differed; the CGB, LGB, and LGB+YC treatments provided 3.9, 7.1, and 7.0 mmol of lactate per dose, with lactate measured as described above for CGB and LGB. In the LGB+YC treatment, a liquid form of LiquiPro SC Drench (Diamond V, Cedar Rapids, IA) was included at the standard dosage for dairy cattle (13 g/d as fed) scaled from predicted 22.7 kg/d of DMI to our DM dosage rate. The LGB and CGB treatments were diluted or dissolved, respectively, in 10 mL of buffer and delivered below the surface of the fermentor contents via syringe. The impeller was temporarily halted during dosing to ensure that treatments did not immediately wash out. The dosage procedure for ^{15}N -enriched $(\text{NH}_4)_2\text{SO}_4$ and the sampling schedule were conducted as in trial 1.

Sample Collection and Analysis

Trial 1. Because gas measurement requires a closed system, fermentor pH was taken over time at h 0, 4, 6, 8, and 12 on d 3 and 4. Filtrate samples were taken on d 7, 8, and 9 directly from the fermentors at 0, 0.5, 1, 1.5, 2, 4, and 6 h postfeeding and were immediately acidified with 6 N HCl at 10% vol/vol and stored at -20°C for several analyses, including VFA, $\text{NH}_3\text{-N}$, $^{15}\text{NH}_3$ enrichment, and lactic acid. Effluent was collected and kept on ice (4°C) for 24 h on d 7, 8, and 9 before being weighed and subsampled on d 8, 9, and 10. A 50-mL sample was acidified with 3 mL of 6 N HCl and immediately stored at -20°C . A 450-mL sample was preserved with 50 mL of formalin and immediately refrigerated for future processing and analysis for ^{15}N enrichment of bacteria. A subsample of 20% of the total effluent was immediately frozen at -20°C with no preservative for digestibility analyses. Two samples were taken for assessment of microbial populations: a 10-mL sample was preserved in 5 mL of formalin for protozoal counting (Dehority, 1993), and a 10-mL sample with no preservative was immediately frozen at -80°C for 16S rRNA gene sequencing.

Acidified samples were initially pooled by fermentor at each time point within period and then centrifuged

at $15,000 \times g$ for 15 min (4°C). The supernatant was filtered through a Whatman no. 1 filter (Whatman International, Maidstone, UK). Subsamples of this supernatant were then taken for analysis of VFA, $\text{NH}_3\text{-N}$, $^{15}\text{NH}_3$ enrichment, and lactic acid. Concentrations of VFA were determined using GC (Harvatine et al., 2002). The $\text{NH}_3\text{-N}$ concentrations were measured using a standard colorimetric assay (Chaney and Marbach, 1962). Using a diffusion protocol (Hristov et al., 2001), $\text{NH}_4\text{-N}$ was analyzed for ^{15}N enrichment by ion ratio MS (Marine Biology Laboratory, Woods Hole, MA). Lactic acid was determined using a DL-lactic acid colorimetric assay per kit instructions (R-Biopharm AG, Darmstadt, Germany). Concentrations of VFA, $\text{NH}_3\text{-N}$, and lactic acid were determined in the effluent; using recorded effluent outflow, we determined net production of VFA, $\text{NH}_3\text{-N}$, and lactic acid for each fermentor period before statistical analysis.

Protozoal samples were pooled across days by fermentor within period. The combined samples were counted and classified according to Dehority (1993) and divided into small (length $<100 \mu\text{m}$) and large (length $>100 \mu\text{m}$) entodiniomorphids (primarily genus *Entodinium*), *Dasytricha*, and *Isotricha*.

Frozen samples (stored at -80°C) were analyzed using next-generation sequencing (MiSeq platform, Illumina, San Diego, CA) of 16S recombinant DNA using repeated bead beating and the column method (Yu and Morrison, 2004) and purification (QIAamp DNA Stool Mini Kit protocol; Qiagen Inc., Valencia, CA) as outlined by Faulkner et al. (2017) for subsequent analysis of relative sequence abundance within bacterial and archaeal communities.

The frozen sample of 20% of the total effluent from each sampling day was composited across days within fermentor by period and dried at 55°C to determine nutrient digestibilities. Following procedures described by Van Soest et al. (1991), NDF was determined by reflux unit using heat-stable α -amylase (Ankom Technology, Macedon, NY) and sodium sulfite (Ankom Technology). A subsample of dried effluent was further dried at 105°C overnight and then subsequently ashed at 550°C overnight to determine DM and OM, respectively (AOAC, 1990). A subsample of dried effluent was also sent for starch analysis (Hall et al., 2001), which was graciously done in the laboratory of M. B. Hall (US Dairy Forage Research Center, Madison, WI).

To attain an isolated bacterial sample for ^{15}N enrichment analysis, a subsample of effluent (liquid) was first pooled by fermentor across days within period. This composited sample was processed via differential centrifugation using a slightly modified procedure from Noftsger et al. (2003). Samples were first centrifuged at $1,000 \times g$ for 10 min (4°C), and the supernatant

was centrifuged at $15,000 \times g$ for 15 min (4°C). After 2 washings in 0.9% saline and recentrifugations ($15,000 \times g$ for 15 min, 4°C), the bacterial pellet was recovered and lyophilized. Dried effluent samples were alkalized (pH >10) and dried in a sand bath at 55°C to evolve NH_3 (Firkins et al., 1992). The freeze-dried bacterial samples and NAN samples were submitted for analysis of ^{15}N enrichment by ion ratio MS as described previously.

Trial 2. Filtrate samples were taken directly from the fermentor on d 8 and 9 at 0, 0.5, 1, 1.5, 2, 4, 8, and 12 h postfeeding. Effluent was sampled, stored, processed, and analyzed as in trial 1 except that pH was recorded at each hour from 0 to 9 h and 12 h immediately after sampling and included d 7. Protozoal samples were also pooled across days within period by fermentor and then classified as in trial 1, except that remaining protozoa, including the obscure entodiniomorphid genus *Charonina* (Wenner et al., 2018), were grouped as other protozoa. Samples for analysis of ^{15}N enrichment were processed as described in trial 1 but were sent for analysis by ion ratio MS (Delta V Advantage isotope-ratio mass spectrometer; ThermoFinnigan MAT GmbH, Bremen, Germany) by Chanhee Lee (The Ohio State University, Ohio Agricultural Research and Development Center, Wooster).

Statistical Analysis

Data from both trials were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). In 4×4 Latin square designs, period was treated as a random effect, but treatment and fermentor were treated as fixed effects. For samples taken over time, repeated measures were assessed using the compound symmetry and heterogeneous first-order autoregressive covariance structures for the models for trials 1 and 2, respectively. These were determined based on lowest Bayesian information criterion after considering compound symmetry, heterogeneous compound symmetry, and first-order autoregressive, heterogeneous first-order autoregressive, and unstructured error structures.

In trial 1, main effect means of grain source, treatment (LW or SBM), and their interaction were assessed by ANOVA. When interactions of these contrasts with time were $P \leq 0.10$, an ANOVA was performed at each sampling time. The effect of day was included as a random effect in the analysis of daily CH_4 production and H_2 emission data after consideration of daily variations in headspace gas, as reported by Wenner et al. (2017). Relative abundance data are reported here without transformation based on similar determinations and justifications as outlined by Faulkner et al. (2017). Relative abundance $<0.5\%$ for any operational

taxonomic unit was excluded from analysis. In trial 2, treatment means were separated by least significant difference protected by a significant F -test for treatment. When treatment $\times$ time interactions were $P \leq 0.10$, the same mean separation procedure was done at each time. In both trials, significance was determined at $P \leq 0.05$ and a trend was indicated by $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Trial 1

We observed no differences or trends ($P > 0.10$) in either NDF or OM digestibilities; however, there was a trend ($P = 0.09$) toward greater starch digestibility for corn than for wheat (Table 2). Starch digestibility was high ($>97.0\%$) compared with a mean of 87.7% reported in a meta-analysis of continuous culture studies (Hristov et al., 2012), probably because feeds sedimented more when we slowed impeller speed to retain protozoa compared with normal operating conditions. The sedimentation also might have negated any difference in starch degradability associated with grain source; although not measured, the particle size of wheat appeared visually larger than that of corn, apparently lessening the starch digestibility of wheat a bit more than that of corn.

A trend ($P = 0.07$) for the main effect of addition of LW (+LW) to increase $\text{NH}_3\text{-N}$ production was present (Table 2), which was expected because the supplemented $\text{NH}_3\text{-N}$ present in LW would be measured as produced $\text{NH}_3\text{-N}$ unless assimilated into bacterial N. Corn increased ($P = 0.02$) the proportion of bacterial N derived from $\text{NH}_3\text{-N}$ compared with the main effect of wheat. Corn protein might have a lower degradability than wheat protein (NRC, 2001). Concentrations of $\text{NH}_3\text{-N}$ taken over time revealed a grain $\times$ LW interaction ($P \leq 0.03$) but no interactions of time ($P > 0.15$); +LW (which contains NH_4^+) increased $\text{NH}_3\text{-N}$ concentration from 1.5 to 4 h postfeeding compared no LW (−LW) for wheat diets only (Supplemental Figure S1; <https://doi.org/10.3168/jds.2017-14358>). We observed no differences ($P > 0.10$) for flows of NAN, bacterial N, or non- NH_3 -nonbacterial N (NANBN). The finding that LW had no determinable effects on the proportion of bacterial N derived from $\text{NH}_3\text{-N}$ suggests that the N provided by LW has similar value to the isonitrogenous SBM control treatment under the conditions of this study.

Exploration of the treatment $\times$ time interaction ($P < 0.05$) revealed that total lactate concentrations were greater ($P < 0.05$) in treatments containing LW up to 1 h postdosing but largely disappeared after that (Figure 1A). Responses were similar for D- or L-stereoisomers

Table 2. Nutrient digestibility and effluent N flows in continuous cultures administered corn- or wheat-based concentrate without (–LW) or with (+LW) Lacto-Whey (LW) twice daily in trial 1¹

Item	Corn		Wheat		SEM ²	P-value ³		
	–LW	+LW	–LW	+LW		Grain	LW	Grain × LW
Digestibility, %								
NDF	51.3	53.6	52.4	52.3	4.4	NS	NS	NS
OM	51.1	49.8	52.1	50.3	3.5	NS	NS	NS
Starch	98.2	98.4	97.1	97.9	0.4	0.09	NS	NS
NH ₃ -N, g/d	0.125	0.215	0.109	0.152	0.035	0.03	<0.01	NS
Bacterial N from NH ₃ -N, %	79.0	87.2	76.5	75.9	3.3	0.05	NS	NS
NAN flow, g/d	0.927	0.905	0.897	0.962	0.097	NS	NS	NS
Bacterial N flow, g/d	0.487	0.487	0.481	0.550	0.063	NS	NS	NS
Non-NH ₃ nonbacterial N flow, g/d	0.440	0.418	0.417	0.411	0.045	NS	NS	NS
Bacterial N, g/kg of OMD ⁴	16.1	16.9	15.6	19.0	3.4	NS	0.05	NS
Bacterial N, g/kg of CHOD ⁵	39.4	40.9	40.1	46.2	7.7	NS	NS	NS

¹Lacto-Whey (Fermented Nutrition Corp., Luxemburg, WI) contains 42.0% ammonium lactate (37.0% lactate) on an as-fed basis. Each +LW dose (twice daily) contained 0.574 g of CP and 6.16 mmol of lactate (1.15 g/d of CP and 12.3 mmol/d of lactate). Soybean meal was added to –LW treatments to make all treatments isonitrogenous.

²Standard error of the mean of simple effects ($n = 4$).

³Main effects of grain source, LW, or their interaction; NS = nonsignificant ($P > 0.15$).

⁴Organic matter apparently degraded.

⁵Carbohydrate apparently degraded (sum of starch degradation and NDF degradation).

(Supplemental Figure S2A and S2B; <https://doi.org/10.3168/jds.2017-14358>). Lactate disappearance was mirrored by an increase in molar proportion of propionate at similar or subsequent times (Figure 1B). Because lactate is more slowly absorbed from the rumen than VFA, the rapid conversion to propionate should enhance removal of protons and keep lactate from accumulating above 5 mM, which is a threshold for acidosis (Aschenbach et al., 2011). Fermentor pH was not affected by treatment (Supplemental Figure S3; <https://doi.org/10.3168/jds.2017-14358>). A main effect of LW ($P \leq 0.03$) was detected for total and L-lactate outflows (Table 3). This value includes lactate dosed plus any lactate produced from dietary carbohydrate and not fermented to VFA; therefore, an increase in lactate was expected. Differences in proportions of D- and L-lactate might be explained by differences in microbial populations (to be discussed subsequently).

Total VFA production did not differ ($P > 0.10$; Table 3) among treatments; the production of acetate, propionate, butyrate, and valerate also did not differ. Trends ($P \leq 0.10$) for LW to decrease acetate and increase propionate molar percentages resulted in a trend for decreased acetate:propionate ratio. Isobutyrate and isovalerate production tended ($P = 0.07$) to be lower for LW. Treatment × time interactions ($P < 0.05$) for molar proportions of branched-chain VFA, propionate, and butyrate were detected. The molar percentage of branched-chain VFA decreased (from 5.16 to 4.88 and 5.03 to 4.77 at 1 and 1.5 h postfeeding, respectively; SEM = 0.2; $P < 0.01$) moderately with LW supplementation at 1.0 and 1.5 h postfeeding (data not shown).

Observed decreases in branched-chain VFA directly after dosing support the literature reporting lactate behaving as sugars do (Firkins et al., 2006); responses tend to be more beneficial when starch degradability is moderate (Firkins et al., 2008). The treatment × time interaction for molar percentage of butyrate was more affected by grain source, increasing from 12.6 to 14.3 (SEM = 0.02; $P = 0.02$) with the corn diet 1.5 h postfeeding (data not shown). Although sugars tend to increase butyrate or valerate (Oba, 2011), perhaps through lactate as an intermediate, the responses here tend to support lactate conversion to propionate. Bacteria respond to increasing lactate production by increasing populations of lactilytics (including different strains of the same species) or through allosteric regulation or changes in gene expression; whether through propionate or longer VFA might depend on glucose availability and pH, which were not affected in our study (Nagaraja and Titgemeyer, 2007). Increased propionate supports a gluconeogenic effect of LW, which should help improve dairy cattle transitioning from parturition to peak production (Aschenbach et al., 2010).

A grain × LW interaction ($P = 0.05$; Table 3) was detected such that the combination of wheat and LW decreased ($P = 0.05$) daily H₂ emission compared with all other treatments. In addition, there was a main effect of LW to decrease ($P < 0.01$) daily production of CH₄. Lactate conversion to propionate incorporates reducing equivalents diverted away from H₂ needed for methanogenesis compared with fermentation through acetyl CoA (Ungerfeld, 2013). Decreased methanogenesis also was associated with increased propionate and

Figure 1. Concentration of total lactate and molar percentage of propionate in continuous cultures after being administered either a corn- or wheat-based concentrate without (–LW) or with (+LW) Lacto-Whey (LW; Fermented Nutrition Corp., Luxemburg, WI) twice daily in trial 1. Lacto-Whey contains 42.0% ammonium lactate (37.0% lactate) on an as-fed basis. Each dose (twice daily) had 0.574 g of CP and 6.16 mmol of lactate, and –LW treatments had an isonitrogenous dosage of soybean meal. Main effects of grain source or its interaction with time were $P > 0.15$. (A) For total lactate, there was a main effect of LW ($P < 0.01$) and time ($P < 0.01$) and an LW $\times$ time interaction ($P < 0.01$). The main effect of +LW increased ($P \leq 0.01$; **) lactate compared with –LW at 0, 0.5, and 1 h postfeeding. The pooled SEM for the hourly means was 0.13 mM. (B) For molar proportion of propionate, there was a main effect of time ($P < 0.01$) and an LW $\times$ time interaction ($P = 0.04$). The main effect of +LW tended ($P \leq 0.08$; †) to increase propionate compared with –LW at 1 and 1.5 h postfeeding. The pooled SEM for the hourly means was 0.76 mol/100 mol. Main effects of grain source or its interaction with time were $P > 0.15$ for both panels.

isovalerate in continuous culture (Wenner et al., 2017) and in goats (Denman et al., 2015).

There were no differences ($P > 0.10$) in concentration of protozoa in the effluent, averaging 10,400 cells/

mL (data not shown). Lactate uptake and fermentation occurred primarily in the genus *Entodinium* (Newbold et al., 1987) compared with *Dasytricha* and *Isotricha*, which produce but do not readily use lactate (Williams

Table 3. Net production of fermentation end products in continuous cultures administered corn- or wheat-based concentrate without (–LW) or with (+LW) Lacto-Whey (LW) twice daily in trial 1¹

Item	Corn		Wheat		SEM ²	<i>P</i> -value ³		
	–LW	+LW	–LW	+LW		Grain	LW	Grain × LW
Effluent, L/d	2.88	2.88	2.87	2.85	NA	NA	NA	NA
Lactate, mmol/d								
Total	0.27	0.48	0.25	0.39	0.06	NS	0.03	NS
D-Lactate	0.18	0.23	0.16	0.19	0.05	NS	NS	NS
L-Lactate	0.09	0.25	0.09	0.20	0.02	NS	<0.01	NS
Total VFA production, mmol/d	241	259	236	226	27	0.14	NS	NS
Individual VFA, mol/100 mol								
Acetate	62.5	60.4	61.9	60.7	0.9	NS	0.08	NS
Propionate	19.5	21.2	19.9	21.5	1.0	NS	0.10	NS
Butyrate	13.0	14.0	13.5	13.5	0.8	NS	NS	NS
Valerate	1.75	1.75	1.80	1.79	0.08	NS	NS	NS
Isobutyrate	0.83	0.65	0.81	0.72	0.07	NS	0.01	NS
Isovalerate	2.40	2.03	2.07	1.72	0.3	0.06	0.04	NS
Acetate:propionate	3.24	2.87	3.12	2.83	0.2	NS	0.08	NS
Emission, mmol/d								
H ₂	0.21	0.27	0.30	0.16	0.09	NS	NS	0.05
CH ₄	34.3	24.2	40.0	21.4	8.4	NS	<0.01	NS

¹Lacto-Whey (Fermented Nutrition Corp., Luxemburg, WI) contains 42.0% ammonium lactate (37.0% lactate) on an as-is basis. Each dose (twice daily) had 0.574 g/d of CP and 6.16 mmol/d of lactate; –LW treatments had an isonitrogenous dosage of soybean meal.

²Standard error of the mean of simple effects ($n = 4$).

³Main effects of grain source, LW, or their interaction; NS = nonsignificant ($P > 0.15$); NA = not analyzed statistically.

and Coleman, 1992). Lactolytic entodinia relative to lactolytic bacteria might have been too low for lactate administration to increase protozoal abundance. In the rumen of cattle fed high-grain diets, lactate and protozoal counts tend to be inversely related because of protozoal sensitivity to lower pH and their ability to sequester starch from fermentation (Nagaraja and Titgemeyer, 2007).

Illumina MiSeq 16S rRNA gene sequencing revealed modest effects of grain source and LW on bacterial and archaeal relative abundance (Table 4). The bacterial families were generally consistent in relative abundance with results from in vivo (Golder et al., 2014). We observed no main effect of LW for any of the bacterial families characterized ($P > 0.10$). Compared with wheat, corn increased the relative sequence abundance of some bacterial families such as *Christensenellaceae* ($P = 0.01$), *Lentisphaeria* Uncultured Rumen RFP12 ($P = 0.04$), and *Erysipelotrichaceae* ($P = 0.04$). Changes in ruminal bacterial populations appear to be most affected by chemical composition of the diet and passage rate (Kamke et al., 2016; Derakhshani et al., 2017). Kamke et al. (2016) suggested that *Erysipelotrichaceae* may ultimately play an important role during the transition period from calving to high production, warranting further investigation in relation to LW and other ammonium lactate supplements. Compared with wheat main effects, corn tended ($P \leq 0.10$) to decrease *Bacteroidales* BS11 Gut Group and *Succinivibrionaceae* but tended to increase ($P \leq 0.08$) *Spirochaeta* and

Lentisphaeria Uncultured RFP12. The BS11 group is hemicellulolytic (Solden et al., 2017), and the other taxa degrade starch and ferment sugars (Golder et al., 2014; Deutsch et al., 2017). A grain × LW interaction ($P = 0.03$) was detected such that the combination of LW and wheat increased *Bacteroidales* S24-7. Using diverse substrates, this bacterium appears to couple an NADH dehydrogenase to a respiratory chain (Ormerod et al., 2016) and therefore might be more adept at using the reducing equivalents associated with lactate uptake and metabolism, explaining its increased abundance in cattle induced with rumen acidosis (McCann et al., 2016). Inclusion of LW also tended ($P = 0.07$) to increase *Clostridiaceae*. The lack of many significant treatment differences observed in bacterial populations supports the lack of main effects observed in N flow data (Table 2). Relative abundance of important bacterial groups should be confirmed by quantitative PCR (Firkins and Yu, 2015).

No treatment differences ($P \geq 0.10$) were detected in the relative abundance of methanogens characterized in this study; however, the main effect of wheat tended ($P = 0.10$) to increase *Methanospaera* compared with corn (Table 4). The lack of significant differences in methanogen relative abundance further supports the likelihood that acetate:propionate or methanogen activity can shift rather than relative abundance (Firkins and Yu, 2015), thus explaining the decrease in daily CH₄ production observed with LW supplementation. The relative lack of significant treatment effects across

microbial populations suggests that LW exerts more immediate influence based on providing specific substrates rather than changing microbial populations.

No differences between treatments were revealed in the relative abundance of the characterized bacterial lactilytics in genera *Selenomonas* and *Megasphaera* (Supplemental Table S1; <https://doi.org/10.3168/jds.2017-14358>). The increased relative abundance of *Selenomonas* versus *Megasphaera* species might explain the trend toward propionate production over butyrate production (Nagaraja and Titgemeyer, 2007; Hackmann and Firkins, 2015). D-Lactate flow to the effluent (net production) was not increased by +LW, but L-lactate outflow increased moderately for +LW compared with –LW (Table 3). Characterized *Selenomonas ruminantium* strains use L-lactate, whereas *M. elsdenii* possesses enzymes (e.g., NAD-independent D-LDH) to also use D-lactate (Hino and Kuroda, 1993; Evans and Martin,

1997). Both lactate production and usage are under transcriptional and allosteric control (Hackmann and Firkins, 2015). Thus, the relatively low supplementation rate of lactate (0.85% of diet, DM basis) and rapid rate of disappearance (Figure 1) in this study may explain the unexpected lack of changes to the lactilytic population.

Trial 2

No differences or trends ($P > 0.10$) were observed in NDF or OM digestibilities in trial 2 (Table 5). This finding was expected and is consistent with previous literature, specifically with regard to YC supplementation (Yang et al., 2003). Starch digestibility was not different ($P > 0.10$) and was high (99%) as with trial 1, resulting from sedimentation of starch granules in the fermentor jars.

Table 4. Selected relative abundance of bacterial and archaeal 16S recombinant DNA (% of total 16S recombinant DNA copies) in continuous cultures administered corn- or wheat-based concentrate without (–LW) or with (+LW) Lacto-Whey (LW) twice daily in trial 1¹

Item	Corn		Wheat		SEM ²	P-value ³		
	–LW	+LW	–LW	+LW		Grain	LW	Grain × LW
Bacterial family								
<i>Prevotellaceae</i>	26.1	27.4	25.6	25.8	2.3	NS	NS	NS
<i>Lachnospiraceae</i>	12.4	12.1	13.7	13.2	3.0	NS	NS	NS
<i>Rikenellaceae</i>	11.0	11.6	9.7	11.1	2.9	NS	NS	NS
<i>Bacteroidales</i> BS11 gut group	7.96	6.46	11.27	10.32	2.47	0.10	NS	NS
Unassigned other	7.97	6.53	6.68	7.00	1.03	NS	NS	NS
<i>Succinivibrionaceae</i>	4.40	3.92	6.75	7.25	1.28	0.06	NS	NS
<i>Ruminococcaceae</i>	5.26	5.32	5.60	4.85	0.56	NS	NS	NS
<i>Bacteroidales</i> S24-7	5.75	4.03	5.08	5.48	0.84	NS	0.13	0.03
<i>Spirochaeta</i>	9.18	11.2	5.57	4.48	2.37	0.07	NS	NS
<i>Veillonellaceae</i>	1.69	1.63	1.86	1.85	0.31	NS	NS	NS
<i>Christensenellaceae</i>	1.18	1.13	0.95	0.95	0.15	0.01	NS	NS
Other <i>Bacteroidales</i>	0.914	0.690	0.881	0.746	0.156	NS	NS	NS
<i>Clostridiaceae</i>	0.106	1.047	0.287	1.633	0.705	NS	0.07	NS
<i>Bacteroidales</i> RF16	0.574	0.530	0.603	0.658	0.304	NS	NS	NS
<i>Anaeroplasmataceae</i>	0.565	0.695	0.494	0.578	0.220	NS	NS	NS
<i>Clostridiales</i> family XIII incertae sedis	0.463	0.398	0.514	0.386	0.064	NS	NS	NS
<i>Lentisphaeria</i> uncultured RFP12	0.261	0.322	0.214	0.154	0.056	0.08	NS	NS
<i>Lentisphaeria</i> uncultured rumen RFP12	0.244	0.252	0.228	0.199	0.087	0.04	NS	NS
Other <i>Clostridiales</i>	0.209	0.211	0.216	0.274	0.036	NS	NS	NS
<i>Erysipelotrichaceae</i>	0.217	0.305	0.204	0.159	0.042	0.04	NS	0.08
<i>Bacteroidaceae</i>	0.259	0.233	0.177	0.206	0.041	NS	NS	NS
<i>Fibrobacteraceae</i>	0.162	0.368	0.171	0.161	0.085	NS	NS	NS
<i>Desulfuromonadales</i> GR-WP33–58	0.229	0.223	0.218	0.141	0.048	NS	NS	NS
Archaeal family								
<i>Methanobacteriaceae</i>	0.088	0.076	0.059	0.077	0.027	NS	NS	NS
<i>Methanomicrobiaceae</i>	0	0.004	0.004	0	0	NS	NS	0.14
<i>Methanosarcinaceae</i>	0	0.002	0.001	0	0	NS	NS	NS
Uncharacterized <i>Thermoplasmatales</i>	0.080	0.076	0.028	0.035	0.026	0.04	NS	NS
Vadin CA11 ⁴								

¹Lacto-Whey (Fermented Nutrition Corp., Luxemburg, WI) contains 42.0% ammonium lactate (37.0% lactate) on an as-fed basis. Each dose (twice daily) had 0.574 g/d of CP and 6.16 mmol/d of lactate; –LW treatments had an isonitrogenous dosage of soybean meal.

²Standard error of the mean of simple effects ($n = 4$).

³Main effects of grain source, LW, or their interaction; NS = nonsignificant ($P > 0.15$).

⁴Represents a large assortment of archaea that are not well characterized and made up of predominantly sequences from clone libraries, first classified in sheep (Wright et al., 2004).

Treatment differences were detected in the daily production of $\text{NH}_3\text{-N}$, with the combination of LGB+YC yielding the highest ($P < 0.01$) $\text{NH}_3\text{-N}$ production (Table 6). The LGB treatment yielded the second highest ($P < 0.01$) production, and CGB and CON had the lowest $\text{NH}_3\text{-N}$ production and were not different ($P > 0.10$) from each other. Similar to our findings, Miller-Webster et al. (2002) reported a trend for increased $\text{NH}_3\text{-N}$ concentrations with YC supplementation in continuous culture. In contrast to the current findings, Hristov et al. (2010) reported a trend for decreased $\text{NH}_3\text{-N}$ concentrations when YC was fed to heifers; however, these authors cited studies with variable results. A treatment $\times$ time interaction was detected ($P = 0.03$) such that LGB+YC increased ($P < 0.05$) $\text{NH}_3\text{-N}$ concentration compared with CON and CGB from 0.5 to 4 h post-feeding and dosing (Supplemental Table S2; <https://doi.org/10.3168/jds.2017-14358>). These data are consistent with $\text{NH}_3\text{-N}$ production data and might reflect an ability of YC to slow $\text{NH}_3\text{-N}$ utilization, providing ample N to the microbial populations between meals. In combination, these data provide evidence that LGB and LGB+YC would increase $\text{NH}_3\text{-N}$ concentrations in the rumen more than isonitrogenous supplementation of SBM or CGB, at least under the conditions of this study.

There were no differences in bacterial N derived from $\text{NH}_3\text{-N}$ or bacterial N flow (Table 5). A trend for greater ($P \leq 0.10$) NAN flow was observed in the LGB+YC treatment compared with all other treatments. In addition, NANBN tended to be greater ($P = 0.10$) in the LGB+YC treatment compared with CON and CGB. The slight differences in NAN and NANBN flows may represent RUP from undegraded YC or could reflect a role of YC on proteolysis. Yoon and Stern (1996) reported no differences in ruminal $\text{NH}_3\text{-N}$ concentrations or $\text{NH}_3\text{-N}$ flow to the duodenum of cattle, but YC increased cellulolytic and proteolytic counts and decreased their estimate of RUP, which is inconsistent with our trend for increased NANBN flows and the potential for YC to inhibit proteolysis or deamination (Chaucheyras-Durand et al., 2008).

No treatment $\times$ time interactions ($P > 0.10$) were detected for concentrations of total lactate (Figure 2) or either of the stereoisomers (Supplemental Figure S4; <https://doi.org/10.3168/jds.2017-14358>). Similar responses over time indicate racemic mixtures for LGB and CGB. Production of total lactate and D- and L-lactate was greater ($P \leq 0.05$) for CGB, LGB, and LGB+YC compared with the SBM control (CON; Table 6). These production data include the amount dosed; therefore, we expected an increase in the amount

Table 5. Nutrient digestibility and N flows in continuous cultures administered a soybean meal control (CON) or an ammonium lactate product, GlucoBoost (GB; Fermented Nutrition Corp., Luxemburg, WI), administered in crystal (CGB) or liquid (LGB) form without or with (LGB+YC) yeast culture (YC) in trial 2

Item	Treatment ¹				SEM ²
	CON	CGB	LGB	LGB+YC	
Apparent digestibility, %					
NDF	39.9	41.4	39.3	38.5	2.2
OM	40.1	40.8	41.1	39.3	1.4
Starch	99.4	99.4	99.3	99.2	0.1
$\text{NH}_3\text{-N}$, g/d	0.047 ^c	0.053 ^c	0.069 ^b	0.083 ^a	0.008
Bacterial N from $\text{NH}_3\text{-N}$, ³ %	73.5	73.2	85.7	85.3	6.0
NAN flow, g/d	1.11 ^{ab}	1.08 ^b	1.10 ^b	1.20 ^a	0.05
Bacterial N flow, g/d	0.650	0.660	0.618	0.625	0.052
Non- NH_3 nonbacterial N, g/d	0.460 ^b	0.425 ^b	0.485 ^{ab}	0.573 ^a	0.053
Bacterial N, g/kg of OMD ⁴	27.9	27.9	25.7	27.1	2.8
Bacterial N, g/kg of CHOD ⁵	27.6	27.8	26.5	27.0	2.3

^{a-c}Means within a row with different superscripts differ ($P \leq 0.10$).

¹Ammonium lactate in crystal (1.2% of the diet, DM basis) or liquid (2.5% of the diet, DM basis) made isonitrogenous across all treatments. The amount of CP from CGB, LGB, and LGB+YC was 0.177, 0.419, and 0.364 g per each of 2 daily doses, respectively; appropriate amounts of soybean meal were added to bring all treatments, including the control, to 0.419 g of CP per dose. The CGB, LGB, and LGB+YC provided 3.90, 7.06, and 6.96 mmol of lactate per each of 2 doses, respectively.

²Standard error of the mean ($n = 4$).

³Fourth period not included in the statistical analysis of bacterial N derived from $\text{NH}_3\text{-N}$ due to compromised sample integrity.

⁴Organic matter apparently degraded.

⁵Carbohydrate apparently degraded (sum of starch degradation and NDF degradation).

Table 6. Net production of fermentation end products in continuous cultures administered a soybean meal control (CON) or an ammonium lactate product, GlucoBoost (GB; Fermented Nutrition Corp., Luxemburg, WI), administered in crystal (CGB) or liquid (LGB) form without or with (LGB+YC) yeast culture (YC) in trial 2

Item	Treatment ¹				SEM ²
	CON	CGB	LGB	LGB+YC	
Effluent, L/d	2.92	2.90	2.89	2.87	0.02
Lactate, mmol/d					
Total	0.13 ^b	0.39 ^a	0.35 ^a	0.41 ^a	0.04
D-Lactate	0.08 ^b	0.20 ^a	0.18 ^a	0.23 ^a	0.02
L-Lactate	0.05 ^b	0.19 ^a	0.15 ^a	0.18 ^a	0.03
Total VFA production, mmol/d	283	279	278	280	8
Individual VFA, mol/100 mol					
Acetate	65.4 ^a	65.9 ^a	62.2 ^b	62.6 ^b	0.7
Propionate	20.0 ^b	19.3 ^b	21.9 ^a	21.5 ^a	0.6
Butyrate	11.7 ^b	11.8 ^b	13.2 ^a	13.1 ^a	0.5
Valerate	1.40	1.35	1.37	1.35	0.10
Isobutyrate	0.49 ^a	0.50 ^a	0.38 ^b	0.42 ^{ab}	0.04
Isovalerate	1.08 ^a	1.10 ^a	0.90 ^b	1.08 ^a	0.10
Acetate:propionate	3.29 ^a	3.42 ^a	2.85 ^b	2.93 ^b	0.10

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹Ammonium lactate in crystal (1.2% of the diet, DM basis) or liquid (2.5% of the diet, DM basis) made iso-nitrogenous across all treatments. The amount of CP from CGB, LGB, and LGB+YC was 0.177, 0.419, and 0.364 g per each of 2 daily doses, respectively; appropriate amounts of soybean meal were added to bring all treatments, including the control, to 0.419 g of CP per dose. The CGB, LGB, and LGB+YC provided 3.90, 7.06, and 6.96 mmol of lactate per each of 2 doses, respectively.

²Standard error of the mean ($n = 4$).

of lactate within treatments other than the SBM control. The visually prolonged accumulation of total lactate in LGB+YC (Figure 2) coincided with the nadir

for fermentor pH; the main effect mean for LGB+YC was lower ($P \leq 0.01$) than that for CON and LGB and tended to be lower ($P = 0.08$) than that for CGB (Sup-

Figure 2. Measured total lactate concentration after feeding and dosing continuous cultures either a soybean meal control (CON) or an ammonium lactate product, GlucoBoost (GB; Fermented Nutrition Corp., Luxemburg, WI), in crystal (CGB) or liquid (LGB) form without or with (LGB+YC) yeast culture (YC) in trial 2. The amount of CP from each dose of CGB, LGB, and LGB+YC was 0.17, 0.43, and 0.36 g per each of 2 daily doses, respectively; appropriate amounts of soybean meal were added to bring all treatments, including the control, to 0.43 g of CP per dose. The CGB, LGB, and LGB+YC provided 3.90, 7.06, and 6.96 mmol of lactate per dose (twice daily), respectively. There was a trend ($P = 0.09$) for a main effect of treatment and a main effect of time ($P < 0.01$), but the treatment $\times$ time interaction was $P > 0.15$. The main effect mean of LGB+YC (0.379 mM) was greater ($P < 0.05$) than the main effect means for CGB and CON (0.115 and 0.195 mM) but not different ($P > 0.15$) from that of LGB (0.195 mM). The pooled SEM was 0.088.

plemental Figure S5; <https://doi.org/10.3168/jds.2017-14358>). The YC addition to LGB apparently delayed lactilysis, in contrast with expectations (Desnoyers et al., 2009). Some authors have noted lower proportions of D-lactate compared with L-lactate within the rumen regardless of diet (Newbold et al., 1987; Møller et al., 1997). There were no differences ($P > 0.10$) in concentration of protozoa in the effluent, averaging 6,902 cells/mL (data not shown). Because protozoal fractions metabolize L-lactate more extensively than D-lactate in vitro (Newbold et al., 1987), the low protozoal numbers in our study might have prolonged L-lactate more than would be expected in vivo.

There were no effects of lactate supplementation (+LW, Supplemental Figure S3; LGB, Supplemental Figure S5, <https://doi.org/10.3168/jds.2017-14358>) on pH in either of our studies. The pH never decreased below 6.1, which is above the typical cutoff for inhibition of most ruminal microbes (Nagaraja and Titgemeyer, 2007). Lactate-fermenting bacteria are able to function at a low pH; therefore, lactate should not accumulate and contribute significantly to ruminal pH depressions associated with SARA (Nagaraja and Titgemeyer, 2007). Krause and Oetzel (2005) reported low, stable lactate concentrations during a SARA challenge, concluding that lactate was not the main cause of low ruminal pH.

No treatment $\times$ time interactions ($P > 0.10$) were detected for total VFA or any of the individual VFA measured (data not shown). Daily total VFA production exhibited no differences ($P > 0.10$) between treatments (Table 6). The LGB and LGB+YC decreased ($P < 0.05$) the molar percentage of acetate, whereas propionate and butyrate molar percentages increased ($P < 0.01$) compared with CON and CGB. Liquid GB and LGB+YC decreased ($P < 0.05$) acetate:propionate compared with CON and CGB. Increases in propionate and butyrate support one of our hypotheses: increased lactate conversion to propionate and butyrate, as discussed previously for trial 1. Although GB in its liquid form increased bacterial N from $\text{NH}_3\text{-N}$ and decreased acetate:propionate, results from dissolved CGB were consistently most similar to CON, probably because of its lower lactate dose but also potentially because the crystallization process with Ca (Marriott, 1985) might have slowed the release of lactate for fermentation to VFA.

CONCLUSIONS

The observed trend for increased propionate production in the first 2 h postfeeding and dosing in trial 1 and the increased total propionate production in trial 2

support the gluconeogenic benefit of liquid ammonium lactate products, LW and LGB, as passed to effluent (also likely to be absorbed from the rumen) or primarily metabolized to propionate. The lack of negative effects of these ammonium lactate products (except with LBG+YC) on pH supports prior suggestions that modest amounts of lactate supplementation stimulate the background bacterial lactilic activity and therefore should not increase the risk for ruminal acidosis. In trial 1, grain source had more of an effect than LW supplementation on N flows such that corn stimulated bacterial assimilation of $\text{NH}_3\text{-N}$ compared with wheat; however, sedimentation of grain sources within our system warrant repetition of this work in vivo. In trial 2, YC increased $\text{NH}_3\text{-N}$ concentration while also tending to increase NANBN flow. Under the conditions of these 2 studies, LW and GB (in both liquid and crystal forms) supported microbial growth comparable with SBM control. Overall, the results are consistent with our expectation that lactate in LW and GB would be fermented primarily to propionate and that the $\text{NH}_3\text{-N}$ would be efficiently assimilated into microbial N.

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