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Effects of partially replacing dietary corn with molasses, condensed whey permeate, or treated condensed whey permeate on ruminal microbial fermentation

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ABSTRACT

Corn is a feedstuff commonly fed to dairy cows as a source of energy. The objective of this study was to evaluate whether partially replacing dietary corn with molasses or condensed whey permeate, in lactating dairy cow diets in a dual-flow continuous culture system, can maintain nutrient digestibility by ruminal microorganisms. Furthermore, this study evaluated whether treating condensed whey permeate before feeding could aid the fermentation of the condensed whey permeate in the rumen. Eight fermentors were used in a 4 × 4 replicated Latin square with 4 periods of 10 d each. The control diet (CON) was formulated with corn grain, and the other diets were formulated by replacing corn grain with either sugarcane molasses (MOL), condensed whey permeate (CWP), or treated condensed whey permeate (TCWP). Diets were formulated by replacing 4% of the diet dry matter (DM) in the form of starch from corn with sugars from the byproducts. Sugars were defined as water-soluble carbohydrates (WSC) in the rations. The fermentors were fed 52 g of DM twice daily of diets containing 17% crude protein, 28% neutral detergent fiber, and 45% nonfiber carbohydrates. Liquid treatments were pipetted into each fermentor. After 7 d of adaptation, samples were collected for analyses of volatile fatty acids (VFA), lactate, and ammonia, and fermentors' pH were measured at time points after the morning feeding for 3 d. Pooled samples from effluent containers were collected for similar analyses, nutrient flow, and N metabolism. Data were statistically analyzed using Proc MIXED of SAS version 9.4 (SAS Institute Inc.); fixed effects included treatment and time, and random effects included fermentor, period, and square. The interaction of treatment and time was included for the kinetics samples. The TCWP and MOL treatments maintained greater fermentor pH compared with CWP. Total VFA concentration was increased in CWP compared with MOL. The acetate:propionate ratio was increased in TCWP compared with CON, due to tendencies of increased acetate molar proportion and decreased propionate molar proportion in TCWP. Lactate concentration was increased in MOL. Digestibility of WSC was increased in the diets that replaced corn with byproducts. The partial replacement of 4% of DM from corn starch with the sugars in byproducts had minimal effects on ruminal microbial fermentation and increased pH. Treated CWP had similar effects to molasses.

Key words: carbohydrates, lactose, molasses, starch

INTRODUCTION

Dairy nutritionists traditionally classify carbohydrates into structural and nonstructural carbohydrates (NRC, 2001). Nonstructural carbohydrates include starches and sugars found inside the plant cell. Starch can compose up to 70% of the DM found in corn grain (Ferraretto et al., 2013), a common feedstuff fed in cattle diets. Starch increases the energy density of the diet, which, when properly formulated, can allow for improved production (Huntington, 1997). In dairy diets, starch recommendations range from 20% in a dry cow diet to greater than 35% in a lactating cow diet (Grant, 2019). A possible downfall of feeding high-starch diets is that it may reduce NDF digestibility (Firkins et al., 2001; Ferraretto et al., 2013) because it can reduce ruminal pH, favoring against cellulolytic bacteria (Russell and Wilson, 1996), and may lead to ruminal acidosis (Stone, 2004). A potential strategy to prevent reductions in NDF digestibility is to partially

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replace starch with other forms of nonstructural carbohydrates such as readily fermentable carbohydrates in the form of sugar sources (Heldt et al., 1999; Broderick et al., 2008).

Molasses and whey are byproducts of sugar and cheese production, respectively, and are widely used in ruminant diets. Whey permeate is produced by the removal of protein and other solids from whey by ultrafiltration, and it can be condensed through partial water removal. As fed, molasses is composed of about 50% sucrose (Nikodinovic-Runic et al., 2013), whereas whey permeate is composed of about 5% lactose (Cassano et al., 2019). Both molasses and condensed whey permeate can be added as a partial replacement for corn grain, ranging from 3 to 6%, in dairy cattle diets as a source of economical alternative feedstuffs without compromising cow productivity (Baurhoo and Mustafa, 2014). Additionally, the inclusion of dietary sugars has been shown to maintain nutrient digestibility of DM, OM, NDF, and starch (Penner and Oba, 2009), increase molar proportion of butyrate (Chamberlain et al., 1993), and improve microbial protein production (Ribeiro et al., 2005) without decreasing ruminal pH. Sugars can be included at 1.5 to 8% of DM in lactating dairy cattle diets (Firkins et al., 2001; de Ondarza et al., 2017). Although a few studies have shown that sucrose and lactose may be included in dairy diets to maintain production in lactating cows (DeFrain et al., 2006; Broderick et al., 2008), little research has been conducted on inclusion of condensed whey permeate and treated condensed whey permeate.

Due to lactose crystallization, hard crystal deposits could be found at the bottom of holding tanks when storing condensed whey permeate. Treatment of condensed whey permeate with a caustic agent aims to improve shelf life as a result of stabilization, preventing crystallization of the lactose in condensed whey. This stabilization of the structure of lactose crystals in their uncrystallized form and the introduction of sodium hydroxide into the permeate may prevent reductions in ruminal pH and aid fermentation of condensed whey permeate in the rumen (Harris and Mostyn, 2012). The condensed whey permeate had a lighter color and visually seemed to have more deposition at the bottom, whereas the treated condensed whey permeate had a darker color and had visually less deposition at the bottom of the container.

The objective of this experiment was to evaluate whether the partial replacement of corn grain with molasses or condensed whey permeate in a dual-flow continuous culture system can help maintain nutrient digestion of a lactating cow diet by mixed ruminal microorganisms. Furthermore, we aimed to evaluate whether treating condensed whey permeate with sodium hydroxide before feeding could increase nutrient digestibility and use by mixed ruminal microorganisms. We hypothesized that partial replacement of corn starch with sugars from molasses byproducts or condensed whey permeate would maintain adequate ruminal microbial fermentation through maintenance of pH and nutrient digestibility in the ruminal fluid and favorable formation of end products such as VFA. Additionally, we hypothesized that treatment of condensed whey permeate with sodium hydroxide would help prevent reductions in pH and improve its effects on fermentation and increase its rate of fermentation by mixed ruminal microorganisms through prevention of crystallization of the lactose in condensed whey permeate.

MATERIALS AND METHODS

The University of Florida (Gainesville) Institutional Animal Use and Care Committee approved all the procedures for animal care and handling required for this experiment.

Experimental Design and Diets

Eight dual-flow continuous culture fermentors were used in a replicated 4 × 4 Latin square design, with individual fermentors as the experimental units. The experiment consisted of 4 fermentation periods of 10 d each, consisting of 7 d of adaptation and 3 d of sampling. Sequences of 4 experimental diets, 1 per period, were randomly assigned to fermentors. The diets each included different sources of carbohydrates (Table 1). The control diet (**CON**) was formulated with corn grain, whereas the other diets were formulated by replacing a percentage of corn grain with either sugarcane molasses (**MOL**; 6.58%), condensed whey permeate (**CWP**; 4.37%), or treated condensed whey permeate (**TCWP**; 4.61%). The diets were formulated for similar nutrient composition, differing only in the source of carbohydrate, as either starch or water-soluble carbohydrates (**WSC**; sugar). The starch from corn in the control diet was partially replaced, with 50% of sugar coming from either molasses, condensed whey permeate, or treated condensed whey permeate, based on the WSC concentrations of the byproducts. The replacement factor of 50% of sugar coming from each byproduct allowed the inclusion rate of the sugars to be within 1.5 and 8% of DM, an acceptable rate for inclusion of sugars in the dairy cow diet. The condensed whey permeate was treated with 0.5% by weight of sodium hydroxide to 32% by weight of lactose, to raise the pH from below 8 to 8, reducing the crystallization of lactose, a procedure developed to improve its shelf life (Harris and Mostyn,

Table 1. Ingredient and chemical composition of the experimental diets

Item, % of DM	Diet[1] CON	MOL	CWP	TCWP
Ingredient composition				
Corn silage	41.7	41.7	41.7	41.7
Alfalfa hay	18.0	18.0	18.2	18.2
Soybean meal	8.0	8.0	8.0	8.0
Canola meal	8.0	8.0	8.0	8.0
Corn grain	22.0	15.4	17.4	17.2
Molasses	—	6.58	—	—
Untreated condensed whey permeate	—	—	4.37	—
Treated condensed whey permeate	—	—	—	4.61
Mineral premix	1.45	1.45	1.45	1.45
Calcium carbonate	0.55	0.55	0.55	0.55
Urea	0.32	0.32	0.32	0.32
Chemical composition[2]				
OM	92.8	92.3	92.5	92.4
CP	17.1	16.8	16.9	16.9
RDP[3]	10.9	10.8	10.9	10.9
RUP[3]	6.20	6.00	6.00	6.00
NDF	28.7	28.2	28.4	28.4
ADF	17.5	17.3	17.5	17.5
NFC	44.8	45.1	44.9	44.9
NSC	35.2	35.1	35.9	35.8
Starch	30.9	26.9	27.7	27.6
WSC[4]	4.30	8.17	8.22	8.23
Ether extract	2.33	2.21	2.25	2.23
NE_L, Mcal/kg of DM[3]	1.59	1.58	1.59	1.52

[1] CON = control; MOL = molasses; CWP = untreated condensed whey permeate; TCWP = treated condensed whey permeate.

[2] Expressed as % of DM unless otherwise stated.

[3] Estimated using the NRC (2001) model.

[4] Water-soluble carbohydrates (Deriaz, 1961).

2012). Experimental diets were formulated according to NRC (2001) recommendation for a lactating Holstein cow with 680 kg BW, producing 45 kg of milk per day with milk fat, protein, and lactose percentages of 3.5%, 3.0%, and 4.8%, respectively.

Nutrient composition of dry feed ingredients was determined in samples ground through a 1-mm screen in a Wiley mill (model no. 2, Arthur H. Thomas Co.) and sent for analysis to Rock River Laboratories (Watertown, WI). Samples were analyzed for DM (Shreve et al., 2006); ash (AOAC, 1990, method 942.05); NDF and ADF (analyzed sequentially using the Ankom system; Schlau et al., 2021; Ankom Technology, 2014, Appendix A, ADF method), with ash and with heat-stable α-amylase and sodium sulfite for NDF; starch (Hall, 2009); WSC, with sucrose used as the standard to develop the calibration curve and a known hay and corn silage run in each batch to check the accuracy of the curve (Dubois et al., 1956); crude fat (modified AOCS Am5-04; AOCS, 2017); and CP (AOAC International, 2000, method 990.03). Liquid feed samples were analyzed using the same methods at Rock River Laboratories, and partial chemical composition is presented in Table 2. The experimental diets were formulated based on chemical composition of individual feed ingredients. Corn silage was dried for 72 h in a 60°C forced-air oven; then it was ground to 2 mm and used in the diets. Soybean meal and corn grain were ground to 2 mm. Alfalfa hay and canola meal were included as pellets, as purchased, in the diets. Each fermentor was fed the corresponding diet, consisting of 106 g of DM per day, including dry and liquid feed divided into 2 feeding times, at 0800 and 1800 h. Liquid feeds (molasses and condensed whey permeate) were measured and dosed into each corresponding fermentor at each feeding time using a needleless syringe.

Dual-Flow Continuous Culture System Operation

A dual-flow continuous culture system, as developed by Hoover et al. (1976) and described by Arce-Cordero et al. (2021b) and Wenner et al. (2021), was used for this experiment. The fermentation vessels used had an average volumetric capacity of 1.82 L when filled until the solid effluent outflow port. To stimulate fermentation by microorganisms, artificial saliva was infused continuously (Weller and Pilgrim, 1974) at a rate of 3.1 mL/min, with partial removal of fermentation liquid effluent at 1.55 mL/min, which allows the removal of liquid and solid effluents to be controlled at a rate of 11% and 5.5%/h respectively. Constant infusion of N_2 gas maintained an anaerobic environment (200 mL of N_2/min), and constant temperature (39°C) and agitation (100 rpm) were maintained throughout.

Two ruminally cannulated Holstein cows consuming a diet (DM basis: 60% whole-plant corn silage, 12.5% ground corn, 13% citrus pulp, 12% soybean meal, and 2.5% mineral and vitamin mix) similar to the control diet were used as ruminal content donors. Ruminal contents were collected from both cows approximately 1 h after feeding and filtered through 4 layers of cheesecloth into prewarmed flasks (Thermos) for transportation to the laboratory. In the laboratory, each fermentor was prewarmed and infused with N_2 gas before inoculation. Each fermentor was inoculated with a 50:50 mix (vol/vol) of the ruminal fluid collected from both cows until the incubated rumen fluid level reached the solid effluent outflow. On d 5 of each period, artificial saliva was exchanged for ^{15}N-enriched saliva, with 1.54 g of ammonium sulfate ^{15}N per 20 L, to replace 0.71 g of urea. To create a steady state of ^{15}N before changing the saliva, a pulse dose of labeled ammonium sulfate provided 0.1733 g of 10.2% excess $(^{15}NH_4)_2SO_4$ (Sigma-Aldrich) per fermentor. Ammonium sulfate was continuously added to the system as a marker in the artificial saliva, at a rate of 0.077 g/L, until the end of each experimental period. On d 7 the solid and liquid effluent containers were placed in an ice bath at 1°C to prevent any further microbial fermentation. At the end of d 10, allowing for 3 d of collections, the fermentors were disassembled, cleaned, and reassembled for the following period.

Table 2. Partial chemical composition of corn grain versus sugar-containing byproducts used in the experimental diets testing the substitution of starch with sugars in a dual-flow continuous culture system

Chemical composition[1]	Ingredient			
	Corn	Molasses	CWP[2]	TCWP[3]
DM	92.9	69.8	32.3	29.5
CP	8.09	4.03	3.10	3.34
Starch	68.1	7.48	ND[4]	0.79
WSC[5]	2.07	60.8	91.7	86.9
Ether extract	2.20	0.29	0.31	ND
Ash	1.51	10.6	9.35	10.1

[1] Expressed as % DM unless otherwise stated.

[2] CWP = condensed whey permeate.

[3] TCWP = condensed whey permeate treated with a caustic agent.

[4] ND = not detected.

[5] Water-soluble carbohydrates (Deriaz, 1961).

Collection of Data and Samples

Fermentor pH was measured at 0, 1, 2, 4, 6, 8, and 10 h after feeding from each fermentor on d 8, 9, and 10, and samples were collected from inside the fermentors and filtered through 4 layers of cheesecloth. One 10-mL sample was immediately mixed with 0.1 mL of 50% sulfuric acid for later VFA and NH_3-N analyses. Another sample, approximately 1 mL of filtered fluid, was collected for lactate analysis. All samples were stored at −20°C for later processing.

Background samples of homogenized liquid and solid effluents from each fermentor and artificial saliva were collected on d 5. Samples were stored at −20°C for analysis of DM, ash, and ^{15}N abundance. From d 7 to 10, before morning feeding, both liquid and solid effluent containers were weighed, their contents combined and homogenized using a hand mixer for 30 s, and stored at −20°C for subsequent analysis of DM, ash, NDF, starch, WSC, and ^{15}N abundance. Pooled samples representing the whole day of fermentation for VFA, NH_3-N, and lactate were also collected from the homogenized sample by filtering through 4 layers of cheesecloth. Representative digesta and pool samples were collected in the mornings to represent 24 h of fermentation for the previous day.

At the end of d 10, a bacterial pellet was harvested from the total contents of each fermentor through 3 consecutive centrifugations, as outlined by Krizsan et al. (2010). Fermentor contents were blended for 30 s using a blender with 200 mL of saline solution (0.9% NaCl), squeezed through 4 layers of cheesecloth, and then rinsed with another 200 mL of saline solution. The filtered sample was centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments) at 1,000 × *g* for 10 min at 4°C, and the supernatant, free of feed particles, was collected for the next centrifugation at 11,250 × *g* for 20 min at 4°C to isolate the bacterial pellet. The supernatant was discarded, and the bacterial pellet was resuspended in 200 mL of McDougall's solution to purify the pellet. The final centrifugation was performed at 16,250 × *g* for 20 min at 4°C. The bacterial pellet was transferred to a new container and stored at −20°C to be freeze-dried. Dry matter, ash, total N, and ^{15}N abundance were analyzed from the bacterial pellet. All background, digesta, and bacterial samples were freeze-dried and ground using a mortar and pestle 24 h after freeze-drying was complete.

Chemical Analyses

For VFA and NH_3-N analyses, ruminal fluid samples were thawed at room temperature and centrifuged at 10,000 × *g* for 15 min at 4°C, and the supernatant was collected for analysis. Samples for NH_3-N concentration were analyzed using the method described by Broderick and Kang (1980). The supernatant sample collected from the initial centrifugation was analyzed in a 96-well flat-bottom plate using a phenol-hypochlorite solution. After initial centrifugation, VFA samples were further processed following the Ruiz-Moreno et al. (2015) method. A crotonic and metaphosphoric acid solution was added to the supernatant at a 1:5 ratio and allowed to freeze overnight. The samples were later thawed and centrifuged at 10,000 × *g* for 15 min at 4°C, and ethyl acetate was mixed into the supernatant in a 2:1 ratio, vortexed, and allowed to settle. The top layer was transferred to a chromatography vial for analysis. Analysis of total and individual VFA was conducted using gas chromatography (Agilent 7820A GC, Agilent Technologies) with a flame ionization detector and a capillary column (CP-WA × 58 FFAP 25 m 0.53 mm, Varian CP7767, Varian Analytical Instruments) maintained at 110°C, with injector temperature at 200°C and detector at 220°C. Lactate was determined using a kit (R-Biopharm) based on the procedure of Niederholtmeyer et al. (2010).

Diet ingredients and freeze-dried digesta samples were analyzed for DM (AOAC, 1990; method 930.15) and ash (AOAC, 1990; method 942.05); NDF was analyzed (Van Soest et al., 1991; Schlau et al., 2021) with heat-stable α-amylase and sodium sulfite modified for Ankom 200 Fiber Analyzer (Ankom Technology) for NDF; starch was analyzed using methods described in Hall et al. (2015); WSC was analyzed using methods described in Deriaz (1961); and total N was analyzed (AOAC International, 2000; method 990.03) using rapid combustion with a micro elemental N analyzer (Vario Micro Cube, Elementar). The atomic percentage of ^{15}N was determined using isotope ratio mass spectrometry and reported as the fractional abundance of isotopes $(^{14}N/^{15}N) \times 100$.

About 0.02 g of background, digesta, and bacteria samples were combined with 2.0-mm zirconia beads to be ground using a Precellys 24 Bead Mill Homogenizer (Bertin Instruments) at 5,500 × *g* for 10 s at room temperature for ^{15}N analysis. Based on expected ^{15}N concentrations, 4, 2, and 1 mg, respectively, of ground samples were weighed into 8-mm × 5-mm tin capsules using a microbalance (Excellence Plus XP Micro Balance, Mettler-Toledo GmbH). For complete evaporation of residual NH_3-N, 35 μL of K_2CO_3 (10 g/L) was pipetted into each capsule and placed in an oven at 40°C and allowed to dry overnight (Reynal et al., 2005). For background and bacteria samples, DM, ash, and concentration of total N were analyzed as described previously.

Calculations

Flow of bacterial N and bacterial efficiency were determined as follows:

$$\text{Bacterial N flow (g/d)} = (\text{NAN flow} \times \%\text{ atom excess of }^{15}\text{N of NAN effluent})/(\%\text{ atom excess of }^{15}\text{N of bacteria pellet}).$$

The percent excess of ^{15}N of NAN effluent was obtained by subtracting % atom ^{15}N in the background from the % atom excess of ^{15}N of NAN effluent (Calsamiglia et al., 1996).

Flows of NH_3-N, NAN, and N metabolism were determined using Bach and Stern (1999):

$$\text{NH}_3\text{-N flow (g/d)} = \text{effluent NH}_3\text{-N concentration (mg/dL)}/1{,}000 \times [\text{total effluent flow (g)}/100];$$

$$\text{NAN flow (g/d)} = \text{effluent grams of total N} - \text{effluent grams of NH}_3\text{-N};$$

$$\text{Dietary N flow (g/d)} = \text{effluent grams of NAN} - \text{effluent grams of bacterial N};$$

$$\text{Bacterial efficiency} = \text{grams of bacterial N flow/kilograms of OM truly digested (Calsamiglia et al., 1996)};$$

$$\text{Efficiency of N use} = (\text{grams of bacterial N/grams of available N}) \times 100 \text{ (Bach and Stern, 1999)}.$$

True digestibility of nutrients (OM, CP, NDF, WSC, and starch) was estimated according to Soder et al. (2013):

$$\text{Percent nutrient digestibility (DM basis)} = 100 \times [\text{grams of nutrient intake} - (\text{effluent grams of nutrient} - \text{saliva grams of nutrient} - \text{bacteria grams of nutrient})] \div \text{grams of nutrient intake}.$$

Statistical Analysis

Statistical analysis of data was performed with the MIXED procedure of SAS 9.4 (SAS Institute Inc.). Data were analyzed as a duplicated 4 × 4 Latin square design. The model used for evaluation of kinetics of pH and concentration of NH_3-N, lactate, and VFA over time was analyzed through repeated measures, which included the fixed effects of treatment, time, and interaction of treatment × time, and the random effects of fermentor, period, and square. The effect of experimental treatment was evaluated through multiple comparisons by comparing all treatment means, applying Bonferroni means separation if the treatment was significant. Additionally, mean separation by hour was performed for the kinetics samples. The covariance structure used was the one that yielded the lowest Akaike information criterion for each variable. The 2 most used ones were compound symmetry and autoregressive heterogeneous. The model used for the evaluation of the pooled NH_3-N, VFA, lactate, N metabolism, and nutrient digestibility included the fixed effects of day and treatment, and the random effects of fermentor, period, and square. The comparisons were the same as those used for the kinetics analysis. Results are reported as least squares means ± standard error of the means. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Partial chemical results from the ingredients listed in Table 2 are within the range observed in the literature. Fiber fractions were not included because they are virtually zero in CWP and TCWP. Analysis of WSC is a fairly crude assay, as the phenol-sulfuric acid assay is a nonspecific carbohydrate assay. It is likely that WSC was slightly high in CWP and TCWP, but very few studies have adequately compared those. It is also possible that soluble starch may have overlapped with WSC. Starch accrual following treatment in TCWP was not expected, but it was quite low and may not be of biological significance. This could have been an artifact of the starch assay. For example, it is not unusual to have minor starch values for alfalfa samples. Overall, values were within expected range.

pH and Ammonia

Replacing 4% of diet DM from corn starch with sugars found in byproducts had no significant effect between CON and the sugar treatments, but fermentor pH of MOL and TCWP increased compared with CWP ($P < 0.05$). The average fermentor pH of the CWP diet was 6.07, whereas in MOL and TCWP it was 6.21 and 6.20, respectively (Table 3). No difference in fermentor pH was observed between MOL and CON. Broderick et al. (2008) reported no difference in ruminal pH when sucrose replaced from 2.5 to 7.5 percentage units of corn starch in diets of lactating dairy cows. One possible explanation for the increase in pH seen in MOL compared with CWP could be the ability of microorganisms to use sucrose to make glycogen (Hall and Weimer, 2007). The storage of glycogen slows the rate of fermentation and release of fermentation acids, possibly leading to less depression of pH (Oba, 2011) with inclusion of molasses in the diet compared with condensed whey permeate.

Differences in pH observed between the 2 condensed whey permeate diets could be attributable to differences in the composition of the sugar in between condensed whey permeate and treated condensed whey permeate. Although both condensed whey permeate treatments received the same amount of WSC, they

Table 3. Effects of partially replacing corn grain with sugar containing byproducts on pH and 24-h NH_3N, VFA, and lactate pool in a dual-flow continuous culture system

	Treatment[1]					
Item	CON	MOL	CWP	TCWP	SEM	*P*-value
pH	6.12^{ab}	6.21^{a}	6.07^{b}	6.20^{a}	0.08	0.04
NH_3N, mg/dL	21.57	21.31	20.98	22.41	1.62	0.74
Total VFA, m*M*	91.26^{ab}	89.34^{b}	93.37^{a}	90.89^{ab}	1.61	0.02
VFA, % of total VFA						
Acetate (A)	50.26	51.25	51.59	52.06	0.95	0.07
Propionate (P)	25.38	23.45	24.82	22.68	1.26	0.09
Butyrate	18.07	18.87	17.27	18.63	0.97	0.39
Valerate	2.46	2.46	2.50	2.46	0.12	0.96
Isobutyrate	0.78^{a}	0.74^{ab}	0.70^{b}	0.77^{ab}	0.05	0.03
Isovalerate	2.20	2.19	2.29	2.35	0.48	0.16
Caproate	0.85^{ab}	1.04^{ab}	0.83^{b}	1.06^{a}	0.17	0.03
A:P	2.01^{b}	2.20^{ab}	2.14^{ab}	2.32^{a}	0.12	0.03
Lactate, m*M*	0.20^{b}	0.27^{a}	0.23^{b}	0.23^{b}	0.03	<0.01

[a,b] Means within a row with different subscripts differ ($P \leq 0.05$).

[1] CON = control; MOL = molasses; CWP = untreated condensed whey permeate; TCWP = treated condensed whey permeate.

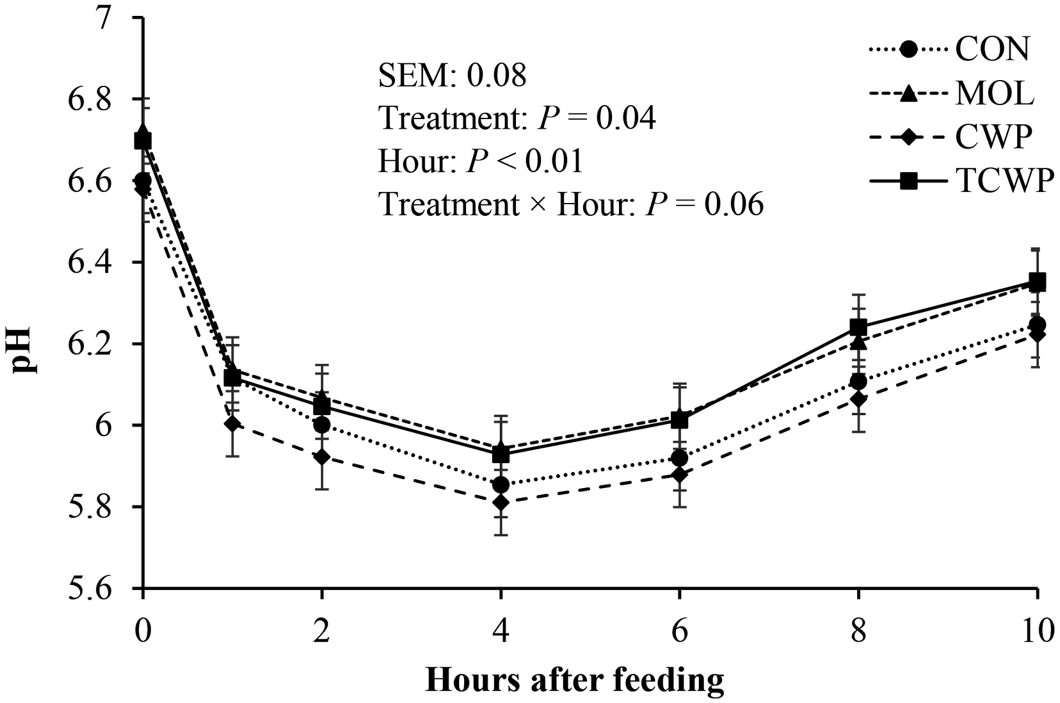

Figure 1. Average pH following feeding of treatments replacing corn with sugar-containing byproducts, and interaction of treatment × hour. Treatments: CON = formulated with corn (starch); MOL = formulated with molasses partially replacing 4% of diet DM of starch from corn with sugar from molasses; CWP = formulated with condensed whey permeate partially replacing 4% of diet DM of starch from corn with sugar from condensed whey permeate; TCWP = formulated with treated condensed whey permeate partially replacing 4% of diet DM of starch from corn with sugar from condensed whey permeate treated with a caustic agent.

were present in different forms due to the caustic agent used in the treated condensed whey permeate. The use of sodium hydroxide for treating condensed whey permeate has been recommended to prevent formation of lactose crystals (Harris and Mostyn, 2012), which may result in slowed fermentation of crystallized compared with uncrystallized lactose. We did not detect such crystals in CWP in this experiment. However, the response of TCWP was more similar to MOL than to CWP in terms of changes in pH, indicating that some change in fermentation characteristics of the treated lactose did occur. In addition to the reported reduction in lactose crystallization related to alkali treatment, it is possible that some portion of the lactose isomerized to lactulose (Zokaee et al., 2002), which could allow different fermentation characteristics in TCWP compared with CWP. However, analysis for lactulose was not performed on the substrates used in the current experiment. Additionally, the presence of NaOH, an alkalizer, in the treated condensed whey permeate could also have prevented the decrease in pH seen in the untreated whey.

We observed a tendency for an effect of treatment by time interaction on pH ($P = 0.06$; Figure 1). It was observed that MOL and TCWP failed to reduce pH and maintain a greater mean fermentor pH compared with CWP. Various studies have shown that lactose can be included in the diet without negative effects on ruminal pH (Schingoethe et al., 1980; DeFrain et al., 2006). However, as seen in this experiment, treatment of condensed whey permeate with NaOH changed its fermentation characteristics, which could have affected the fermentation rate of lactose. Thus, the addition of NaOH could help maintain pH, compared with untreated condensed whey permeate. Additionally, when comparing condensed whey permeate to molasses, the lower concentration of total VFA with molasses did not depress pH compared with condensed whey permeate, which had a greater concentration of total VFA.

No effects in NH_3-N concentration were seen (Table 3). With no differences, it is possible that sugar-utilizing bacteria prefer a different N source for growth (Maeng and Baldwin, 1976). Furthermore, NH_3-N concentration is a net value and is affected by microbial utilization, amino acid use, and amino acid degradation to NH_3-N (Argyle and Baldwin, 1989). Additionally, Hristov et al. (2005) have observed differences in NH_3-N use by microorganisms using glucose or starch. Penner and Oba (2009) also observed no effect in NH_3-N in vivo when 52 early-lactation Holstein cows were fed a high-sugar diet with added sucrose that had 8.4% total sugar, compared with a low-sugar diet that had no inclusion of sucrose and 4.5% total sugar. Vallimont et al. (2004) used a dual-flow continuous culture system to test the effects of partially and completely replacing 7.5% of corn starch with sugar and reported no change in total fermentor NH_3-N concentration. Moreover, in a study by DeFrain et al. (2004), no effect on NH_3-N was seen when liquid whey or pure lactose was added to lactating cow diets. Some studies have reported a decrease in NH_3-N concentration in ruminal fluid when sucrose (Sannes et al., 2002; Broderick et al., 2008) or lactose was added to the diet (DeFrain et al., 2006). Increased concentrations of NH_3-N have also been reported when feeding molasses. A 2-trial study by Broderick and Radloff (2004) reported a quadratic effect on ruminal NH_3-N concentration with the inclusion of molasses in the diets. The lowest NH_3-N concentration was observed with the lowest sugar content at 1.19 mg/dL in one study, and the greatest NH_3-N concentration was observed with a greater sugar content in the second study at 9.30 mg/dL. Although it is expected that sugars will aid the capture of more degradable N (Sniffen et al., 1992), reports have been inconsistent when different sugars were fed. Additionally, more work in fermentors is needed to understand changes in NH_3-N, as there is a lack of exchange of end products in a fermentor compared with in vivo.

Volatile Fatty Acids and Lactate

Data for VFA and lactate concentrations are presented in Table 3. Total VFA concentration was greater in CWP compared with MOL ($P = 0.02$). The results in the present experiment correspond with that of Abdullah et al. (1992), where it was seen in cattle and buffaloes that inclusion of molasses in the diet decreases total VFA concentration. One possible reason for the lower total VFA seen in MOL could be, as discussed earlier, the storage of glycogen in microorganisms. If microorganisms use sucrose to make glycogen, a short-term energy storage (Hall and Weimer, 2007), then this storage delays and slows the production of fermentation acids. With this momentary reduction in fermentation, it is possible that the total VFA concentration could be decreased compared with a treatment that does not have sucrose supplementation, such as CWP. Another potential explanation is the increase in protozoa concentration from supplementing with molasses. Some protozoa are believed to be able to survive 10 d of fermentation in vitro (Hino et al., 1993). Nguyen and Preston (1999) report that supplementation of molasses increased the protozoa population in the rumen of swamp buffaloes, and Mendoza et al. (1993) reported that protozoa-free sheep tended to have greater concentrations of total VFA. Hence, inclusion of molasses in the diet could have increased the protozoa population, leading to a decrease in concentration of total VFA. Protozoa counts were not taken during this experiment; hence, further studies would need to be conducted to test this hypothesis. The results seen in VFA concentrations also correspond to the pH values as expected; Weisbjerg et al. (1998) reported that greater VFA concentration had lower pH, as seen in CWP, and lower VFA concentration had a greater pH, as seen in MOL.

When studying the effects of sugar supplementation on VFA profile, one would expect to observe an increase in the proportion of butyrate (Chamberlain et al., 1993; de Ondarza et al., 2017); this is attributed to the increased production of lactate, due to sugars favoring lactate-utilizing bacteria (Heldt et al., 1999), from which butyrate formation then occurs when the pH of the rumen is around 6.2. However, Baldwin et al. (1962) reported that, as the presence of carbohydrates and lactate in the diet increased, production of propionate by rumen microorganisms increased. In the present experiment, we found no effect on butyrate, in accordance with results seen in Broderick et al. (2008) and Penner and Oba (2009). It is possible that this lack of difference in butyrate concentration could be due to lack of retention of isotrichid protozoa, which have been shown to be important for butyrate production (Hall, 2011). The molar proportion of acetate tended to increase ($P = 0.10$), whereas propionate proportion tended to decrease in TCWP compared with CON ($P = 0.10$). Previous studies have demonstrated that, when sugar was used to replace dietary starch, propionate concentration decreased (Heldt et al., 1999; DeFrain et al., 2004). Without an observed change in butyrate, if the concentration of propionate decreased with TCWP, it can be expected that the proportion of acetate would increase. This can be seen in the acetate:propionate ratio as well. The TCWP treatment had a greater A:P ratio compared with CON ($P = 0.04$). Russell (1998) reported that when pH in incubated rumen fluid decreased from 6.5 to 5.3, the A:P ratio decreased from 1.2 to 0.6. It was seen that 25% of the change in the A:P ratio could be attributed to change in pH. In the current experiment, TCWP, which had a greater pH, tended to have a greater A:P ratio compared with CON, which had a lower pH and lower A:P ratio.

No treatment effects were observed on molar proportion of valerate and isovalerate; however, isobutyrate was lower in CWP ($P = 0.05$) compared with CON. Studies with sugars often do not report the effects on isoacids such as isobutyrate and isovalerate. However, in an in vitro setting it has been observed that, to a

Table 4. Effects of replacement of corn grain with sugar-containing byproducts on digestibility in a dual-flow continuous culture system

	Treatment[2]					
Digestibility,[1] %	CON	MOL	CWP	TCWP	SEM	*P*-value
DM	50.5	50.1	49.9	50.6	1.65	0.97
OM	56.6	56.7	56.2	56.8	1.45	0.97
CP	60.8	59.5	59.0	61.5	2.51	0.67
NDF	48.6	48.2	47.5	48.7	2.46	0.89
Starch	91.6	90.6	90.3	89.6	0.87	0.08
WSC[3]	83.5^{b}	88.7^{a}	91.6^{a}	91.0^{a}	1.19	<0.01

[a,b] Means within a row with different subscripts differ ($P \leq 0.05$).

[1] True digestibility for DM, OM, CP, and dietary starch; apparent digestibility for NDF and WSC.

[2] CON = control; MOL = molasses; CWP = untreated condensed whey permeate; TCWP = treated condensed whey permeate.

[3] Water-soluble carbohydrates (Deriaz, 1961).

lesser extent, these isoacids could increase the rate of cellulose digestion (Bentley et al., 1955). This should be a point of consideration when feeding diets with sugar components, given the concern for decreased NDF digestibility (Hall and Mertens, 2017). Little et al. (1967) tested the effect of valerate, isobutyrate, and isovalerate on cellulolytic digestion in a control medium and reported that valerate had the greatest stimulation on NDF digestibility, followed by isobutyrate, with no effect on digestibility seen with isovalerate. Although we found a decrease in the concentration of isobutyrate in CWP compared with CON, difference in NDF digestibility was observed (Table 4). It is unlikely that a small but significant change in the concentration of these branched-chained VFA would be the cause of a decrease in NDF digestibility. According to Roman-Garcia et al. (2021), greater differences in concentrations of branched-chained VFA would be needed to alter NDF digestibility. Other factors such as dietary starch, diet particle size, and ruminal pH would likely have a greater effect on NDF digestibility.

Caproate is a ketogenic short-chain fatty acid (Kristensen and Harmon, 2005), and its role in ruminal metabolism is not well studied. The molar proportion of caproate was increased with TCWP compared with CWP ($P < 0.01$). Caproate is formed during the secondary fermentation of acetate (Ding et al., 2010); hence, a positive correlation between acetate and caproate exists, which agrees with the numerical pattern of our results. Likewise, a negative relationship exists between propionate and caproate (Ertl et al., 2015). This is also in agreement with results seen in TCWP, which had a lower proportion of propionate and a greater concentration of caproate, whereas CWP, which had a greater proportion of propionate, had lower caproate. More research will be needed to better understand the role of caproate in ruminal metabolism.

Lactate concentration was increased in MOL ($P < 0.05$) compared with the other treatments. We also observed an interaction of treatment and time, as MOL concentration of lactate was greater in the first 4 h after feeding and continued to be greater compared with the other treatments ($P = 0.05$), as shown in Figure 2. Lactate production in the rumen can be increased by the inclusion of soluble carbohydrates (Cullen et al., 1986). Sucrose, the sugar component of molasses, consists of the monomers of glucose and fructose, and it has been shown that glucose and fructose are able to ferment faster than galactose. Glucose and galactose are the monosaccharides that make up the disaccharide lactose, the sugar component of condensed whey permeate. Weisbjerg et al. (1998) tested single doses of sugars in the rumen to estimate their rate of hydrolysis and reported their fermentation rates. As a result of dosing sucrose, a lower ruminal pH was observed compared with when lactose was dosed. With lactose, pH was greater and decreased at a slower rate compared with sucrose; thus, Weisbjerg and colleagues concluded that sucrose was able to ferment faster than lactose. An earlier experiment by Sutton (1968) demonstrated that when equal amounts of glucose, fructose, and galactose were mixed with ruminal contents, after 2 h glucose and fructose are almost completely fermented, whereas galactose was about 55% fermented. This would explain the more rapid fermentation and greater concentration of lactate produced the first 4 h from feeding MOL.

N Flows and Metabolism

Treatments did not affect N flow and metabolism (Table 5). Broderick et al. (2008) did not see improvements in N utilization with the inclusion of sucrose in lactating cow diets. Additionally, DeFrain et al. (2006) saw no change in milk urea N when lactose was given to cows pre- and postpartum. Other studies have reported increases in microbial production with supplementation of molasses. Srinivas and Gupta (1997) reported greater microbial production rate of protein when cattle were given urea-molasses supplements, possibly due to the synergy of N source and energy. Furthermore, Marsetyo et al. (2005) reported that increasing molasses supplementation increased microbial protein synthesis in sheep fed urea-treated barley straw, and no difference in NH_3-N concentration was reported. However, those authors reported increased nitrogen, attributed to more NAN present, thus allowing increased bacterial efficiency due to the increased presence of nitrogen because nitrogen was not limiting. However, in the current study, no positive effects were observed upon microbial protein production or bacterial efficiency related to replacement of the starch from corn with sugars from byproducts.

Nutrient Digestibility

We detected no effects of treatments on digestibility of DM, OM, CP, or NDF (Table 4). Although there are concerns about the replacement of corn grain with sugar-containing byproducts in diets, due to potential of decreased NDF digestibility (Hall and Mertens, 2017), in the current experiment we found no adverse effects on NDF digestibility from starch replacement with sugars. Broderick and Radloff (2004) reported an increase in total-tract digestibility of NDF with molasses supplementation that peaked at a dietary sugar concentration of 7.2% of DM in lactating dairy cow diets. The increase they observed could have been due to their slightly lower dietary sugar total, compared with the 8% used in the current experiment.

Dietary starch digestibility tended to be greater in CON than in other treatments ($P = 0.08$). The WSC digestibility of CON was lower than for the other treatments ($P < 0.01$). Although a basal level of sugars is always present in the diet (Lee et al., 2003), the liquid byproduct inclusion increased WSC concentration for the sugar diets. With more readily fermentable carbohydrates present in the sugar treatments, WSC digestibility could be increased due to greater availability for digestion. Additionally, Hoover et al. (2006) reported that the sugars found in free-sugar-containing byproducts are more digestible than the sugars found in plant-based material. The digestibility levels of WSC in the sugar treatments were all around 90%. It is possible that WSC digestibility was lower with CON because

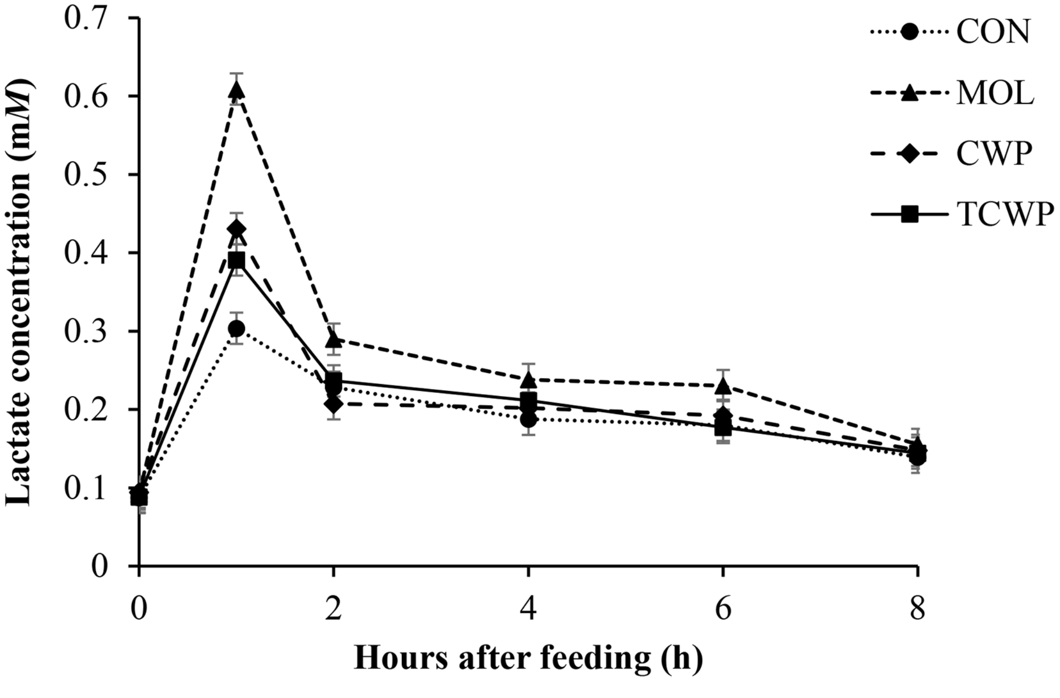

Figure 2. Concentration of lactate after feeding diets that replace corn with sugar-containing byproducts, and interaction of treatment × hour. Treatments: CON = formulated with corn (starch); MOL = formulated with molasses partially replacing 4% of diet DM of starch from corn with sugar from molasses; CWP = formulated with condensed whey permeate partially replacing 4% of diet DM of starch from corn with sugar from condensed whey permeate; TCWP = formulated with treated condensed whey permeate partially replacing 4% of diet DM of starch from corn with sugar from condensed whey permeate treated with a caustic agent.

Table 5. Effects of replacement of corn grain with sugar-containing byproducts on N metabolism in a dual-flow continuous culture system

Item	Treatment[1]				SEM	P-value
	CON	MOL	CWP	TCWP		
N flows, g/d						
Total N[2]	2.92	3.02	2.91	2.92	0.08	0.52
NH_3-N[3]	0.90	0.90	0.87	0.94	0.07	0.71
NAN[4]	2.02	2.12	2.03	1.97	0.06	0.25
Bacterial N[5]	1.00	1.08	0.98	1.02	0.06	0.40
Dietary N[6]	1.02	1.04	1.05	0.99	0.07	0.72
ENU[7]	38.5	42.0	38.1	39.7	2.17	0.35
Bacterial efficiency[8]	18.2	19.6	18.0	17.9	0.89	0.33

[1] CON = control; MOL = molasses; CWP = untreated condensed whey permeate; TCWP = treated condensed whey permeate.

[2] Total n = NH_3-N + NAN (Bach and Stern, 1999).

[3] NH_3-N (ammonia N) = effluent NH_3-N (mg/dL)/1,000 × [total effluent flow (g)/100].

[4] NAN = nonammonia N flow (g/d) = total N − NH_3-N.

[5] Bacterial N flow = (NAN flow × % atom excess of ^{15}N in NAN effluent)/(% atom excess of ^{15}N in bacteria pellet); Calsamiglia et al., 1996.

[6] Dietary N flow (g/d) = NAN − bacterial N.

[7] ENU (efficiency of N use) = (grams of bacterial N/grams of available N) × 100 (Bach and Stern, 1999).

[8] Bacterial efficiency = grams of bacterial N/kilograms of OM truly digested (Calsamiglia et al., 1996).

the sugars in MOL and the condensed whey permeate treatments ferment faster than those present in CON, due to the source of the WSC.

CONCLUSIONS

The partial replacement of corn starch with byproducts that contain either sucrose or lactose in the diet maintained ruminal microbial fermentation and had positive effects on pH. In addition, condensed whey permeate treated with a caustic agent (NaOH) was fermented similarly to molasses by mixed ruminal microorganisms in continuous culture. From helping maintain ruminal pH to maintaining nutrient digestibility, the treatment of sugars warrants more research in the future so that practical levels can be fed to dairy cows.

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