Dietary molasses increases ruminal pH and enhances ruminal biohydrogenation during milk fat depression

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ABSTRACT

Feeding high-concentrate diets has the potential to cause milk fat depression, but several studies have suggested that dietary sugar can increase milk fat yield. Two experiments were conducted to evaluate the ability of dietary molasses to prevent milk fat depression in the presence of a 65% concentrate diet. In trial 1, molasses replaced corn grain at 0, 2.5, or 5% of diet dry matter in diets fed to 12 second-lactation Holstein cows (134 ± 37 d in milk) in a 3×3 Latin square design. Trial 1 demonstrated that replacing up to 5% of dietary dry matter from corn with molasses had positive effects on de novo fatty acid synthesis, increasing the yield of short- and medium-chain fatty acids during diet-induced milk fat depression. Increasing inclusion rate of molasses increased milk fat concentration, but decreased milk yield and milk protein yield. Trial 2 used 7 ruminally cannulated, multiparous, late-lactation Holstein cows (220 ± 18 d in milk) to evaluate effects of dietary molasses on ruminal parameters and milk composition, and also to assess whether increased metabolizable protein supply would alter these responses. Cows were randomly assigned to a dietary treatment sequence in a crossover split plot design with 0 and 5% molasses diets. Dietary treatments were fed for 28 d, with 16 d for diet adaptation, and the final 12 d for 2 abomasal infusion periods in a crossover arrangement. Abomasal infusions of water or AA (5 g of L-Met/d + 15 g of L-Lys-HCl/d + 5 g of L-His-HCl-H₂O/d) were administered 3 times daily for 5 d, with 2 d between infusion periods. Administration of AA had no effect on concentration or yield of any milk components. Addition of molasses increased milk fat concentration (2.71 vs. $2.94 \pm 0.21\%$), but had no effect on yields of milk fat or protein. Dietary molasses decreased total volatile fatty acid concentration (141 vs. 133 ± 4.6 mM), decreased the molar proportion of propionate, and increased the molar proportion of butyrate in ruminal fluid. Molasses also increased ruminal pH (5.73 vs. 5.87 ± 0.06),

decreased the yield of *trans*-10 C18:1, and increased the yield of *trans*-11 C18:1 in milk fat. These data provide evidence that molasses may promote mammary de novo fatty acid synthesis in cows fed high-energy rations by moderating ruminal pH and altering ruminal fatty acid biohydrogenation pathways.

Key words: milk fat depression, molasses, biohydrogenation

INTRODUCTION

Rising feed and operating costs have led dairy producers to seek nontraditional ways to decrease feed costs while maintaining high milk yield. One option that has become more available with the growth of the ethanol industry is distillers grains with solubles (DGS). The readily digestible fiber provided by DGS allows producers to partially replace both forages and concentrates in diets of dairy cattle. However, because of the small particle size, DGS contains little effective fiber; replacing forages with DGS can decrease rumination, chewing activity, and saliva secretion. Kleinschmit et al. (2007) showed that DGS contains only 3.4 to 19.8% physically effective NDF, which is not sufficient to prevent milk fat depression (MFD; Cyriac et al., 2005) unless other sources of effective NDF are included in the diet in adequate quantities. In addition, DGS typically contains 10 to 12% unsaturated fat (Janicek et al., 2008). Dietary unsaturated fatty acids (FA) can slow ruminal fiber digestion (Van Soest, 1994), limit the growth of fiber-digesting bacteria (Jenkins et al., 2008), and contribute to MFD (Schingoethe et al., 2009).

Increasing dietary fiber content in a diet can prevent MFD when feeding DGS, but high-fiber diets limit energy intake and productivity (Allen, 2000). Increasing dietary sugar content may provide an alternative method of preventing MFD from DGS. Molasses is a sugar-containing liquid feed that can increase the ruminal fermentability of dietary carbohydrates, while stimulating DMI (Firkins et al., 2008), serving as a fat carrier, and binding essential nutrients to prevent potential sorting problems (Murphy et al., 1997). Feeding a sugar-based product within a diet can change ruminal fermentation patterns, decrease ruminal NH₃

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concentration (Broderick and Radloff, 2004; DeFrain et al., 2006), and increase ruminal butyrate concentration (Hristov and Ropp, 2003; DeFrain et al., 2006). Sugars undergo rapid fermentation in the rumen, theoretically leading to lactic acid production and decreasing ruminal pH, which potentially could depress fiber digestibility (Oelker et al., 2009). However, Broderick and Radloff (2004) reported that replacing high-moisture corn with molasses resulted in improved fiber digestibility, likely reflecting a stimulatory effect of molasses on fiber-digesting ruminal bacteria. Fiber-digesting bacteria are thought to be primarily responsible for ruminal biohydrogenation of FA (Harfoot and Hazlewood, 1997), suggesting that dietary molasses may be capable of enhancing biohydrogenation of unsaturated FA. Complete biohydrogenation of unsaturated FA eliminates potential negative effects on milk fat synthesis; therefore, molasses may be capable of preventing diet-induced MFD.

The objective of this work was to determine if replacing corn grain with molasses at up to 5% of diet DM would prevent MFD from a high-concentrate ration containing 20% DGS.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Animals, Treatments, and Data and Sample Collection

Trial 1. Twelve second-lactation Holstein cows (696 $\pm$ 52 kg BW, 134 $\pm$ 37 DIM, mean $\pm$ SD) were randomly assigned to square and sequence within square in a replicated 3 $\times$ 3 Latin square design balanced for carryover effects. The intention of the control diet was to induce MFD, and the diet contained 36.6% forage and 21.2% corn dried DGS with 26.2% NDF, 46.4% NFC, and 4.4% ether extract (Table 1). The remaining 2 diets were identical to the control diet except for the inclusion of cane molasses at 2.5 or 5% of diet DM, replacing a portion of the corn grain. The molasses (79 degrees Brix and 71.9% DM) contained no additives. Diets were prepared daily with ground corn, molasses, or both, added to a common base mix representing 95% of diet DM to complete each TMR. Throughout trial 1, cows were housed in a tie-stall facility, milked twice daily (0500 and 1600 h), and fed twice daily (0630 and 1700 h) for ad libitum intake.

Each dietary treatment period lasted 28 d, with 14 d for diet adaptation and 14 d for sample and data collection. During each period, all cows were treated with

Posilac (Monsanto Co., St. Louis, MO) on d 1 and 15. To avoid potential interactions of dietary treatments with the Posilac treatment schedule, feed samples, DMI data, milk yield data, and milk samples were collected on d 16, 19, 22, 25, and 28 of each period. Two milk samples were collected at each milking on these days; 1 sample was used for component analysis and the other was stored at -20°C for analysis of FA profile.

Trial 2. Seven ruminally cannulated, multiparous, late-lactation Holstein cows (220 $\pm$ 18 DIM) were used to evaluate effects of adding molasses, with or without supplemental AA, on ruminal parameters and milk composition. Cows were randomly assigned to a split-plot design with dietary treatments tested as the main plot, and arranged in a switchback design with 3 periods. Dietary treatments were fed for 28 d, with 16 d for diet adaptation. The control diet was formulated to induce MFD, and included 37% forage and 20% corn dried DGS, resulting in a diet with 24.5% NDF, 48.7% NFC, and 4.7% ether extract (Table 2). A portion of the corn grain was replaced with 5% of diet DM as molasses in the treatment diet. The cane molasses used in trial 2 was from the same source as in trial 1 and contained 53% total sugars (DM basis) and 70% DM with no additives. Diets were prepared daily with a base mix of 95% of diet DM with ground corn grain or

Table 1. Ingredient and nutrient composition (% of DM) of experimental diets (trial 1)

Item	Dietary molasses, DM basis		
	0%	2.5%	5%
Ingredient			
Corn silage	24.7	24.7	24.7
Alfalfa hay	11.9	11.9	11.9
Corn DDGS ¹	21.2	21.2	21.2
Ground corn grain	33.9	31.4	28.9
Molasses	—	2.5	5.0
Solvent soybean meal	4.0	4.0	4.0
Expeller soybean meal	2.6	2.6	2.6
Limestone	1.1	1.1	1.1
Trace mineral salt ²	0.4	0.4	0.4
Micronutrient premixes ³	0.2	0.2	0.2
Nutrient			
DM, % as fed	64.3	64.1	63.9
CP	17.4	17.2	17.1
NDF	26.2	26.3	26.3
NFC	46.4	46.3	46.2
Ether extract	4.4	4.4	4.3
Ash	5.5	5.7	5.9

¹Dried distillers grains with solubles.

²Contained 96% NaCl, 0.35% Zn, 0.2% Fe, 0.2% Mn, 0.03% Cu, 0.007% I, and 0.005% Co.

³Included 22% Se premix (600 mg of Se/kg as sodium selenite), 26% magnesium oxide, 6.9% vitamin A premix (30 kIU/g), 17% vitamin E premix (44 IU/g), 1.6% vitamin D premix (30 kIU/g), and 26% 4-Plex (Zinpro Corp., Eden Prairie, MN; contains 2.58% Zn as Zn methionine, 1.43% Mn as Mn methionine, 0.90% Cu as Cu lysine, and 0.18% Co as Co glucoheptonate).

Table 2. Ingredient and nutrient composition (% of DM) of experimental diets (trial 2)

Item	Dietary molasses, DM basis	
	0%	5%
Ingredient		
Corn silage	25.5	25.5
Alfalfa hay	12.6	12.6
Corn DDGS ¹	19.8	19.8
Ground corn grain	33.6	28.8
Molasses	—	4.8
Solvent soybean meal	4.1	4.1
Expeller soybean meal	2.7	2.7
Limestone	1.1	1.1
Trace mineral salt ²	0.4	0.4
Micronutrient premix ³	0.2	0.2
Nutrient		
DM, % as fed	66.4	65.8
CP	16.2	16.1
NDF	24.5	24.5
NFC	48.7	48.6
Starch	36.3	32.9
Sugars (by invertase)	6.4	8.9
Ether extract	4.7	4.6
Ash	5.9	6.2

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³Included 22% Se premix (600 mg of Se/kg as sodium selenite), 26% magnesium oxide, 6.9% vitamin A premix (30 kIU/g), 17% vitamin E premix (44 IU/g), 1.6% vitamin D premix (30 kIU/g), and 26% 4-Plex (Zinpro Corp., Eden Prairie, MN; contains 2.58% Zn as Zn methionine, 1.43% Mn as Mn methionine, 0.90% Cu as Cu lysine, and 0.18% Co as Co glucoheptonate).

molasses added to complete each TMR. Throughout the trial, cows were housed in a tie-stall facility, milked 3 times daily (0500, 1300, and 2400 h), and fed twice daily (0630 and 1500 h) for ad libitum intake.

During trial 1, dietary molasses linearly decreased milk protein yield ($P = 0.03$), resulting in a 7% decrease in milk protein yield for the 5% molasses treatment. These results suggested that MP supply may have limited milk protein synthesis in molasses treatments. Therefore, abomasal infusions of essential AA were tested as a subplot in trial 2 and were arranged as a crossover within each main plot period. During the 28-d dietary-treatment period, the final 12 d were used for 2 abomasal infusion periods. Abomasal infusions of water (1.2 L/d) or AA (5 g of L-methionine/d + 15 g of L-lysine-HCl/d + 5 g of L-histidine-HCl-H₂O/d in 1.2 L of water/d) were administered 3 times daily (0.4 L/dose) for 5 d, with 2 d between infusion periods. Prior to the infusion periods, polyvinylchloride tubing (2.4 mm i.d. Tygon R-3603; Saint-Gobain Performance Plastics Corp., Akron, OH) was passed through the rumen and inserted into the abomasum. A 10-cm rubber flange held the tubing in the abomasum. The end of the tubing extended through the ruminal cannula for administration of AA treatments (Schroeder et al., 2007).

During the final 4 d of each infusion (sub-plot) period, milk yield was recorded and 2 milk samples were collected at each milking; 1 sample was used for component analysis and the second was stored at -20°C for analysis of FA profile. To determine DMI, feed offered and refused were measured for each cow during the final 4 d of each infusion period. Samples of orts, each TMR, and individual feed ingredients were collected and composited by period.

Ruminal contents were collected during each dietary period on d 26 to 28 at 9-h intervals shifted forward 3 h daily to represent every 3 h of a 24-h period. Ruminal digesta samples were collected from 5 locations throughout the rumen and mixed. A portion of each composited sample was frozen at -20°C for microbial population analysis, and a second subsample was strained through 4 layers of cheese cloth to separate fluid from solids. The ruminal fluid was placed in a 20-mL vial, pH was measured using a portable pH meter (Orion Research Inc., Boston, MA), and then the sample was frozen at -20°C for VFA and NH₃ analyses.

Sample Analysis

One milk sample from each collection was used to determine the concentration of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN), MUN (MUN spectrophotometer, Bentley Instruments Inc.), and SCC (SCC 500, Bentley Instruments Inc.) by Heart of America DHIA (Manhattan, KS). The 3.5% FCM was calculated as $0.434 \times \text{milk yield} + 16.216 \times \text{milk fat yield}$; ECM was calculated as $0.327 \times \text{milk yield} + 12.86 \times \text{fat yield} + 7.65 \times \text{protein yield}$ (Dairy Record Management Systems, 2009); and SCM was calculated as $12.3 \times \text{fat yield} + 6.56 \times \text{SNF yield} - 0.0752 \times \text{milk yield}$ (Tyrrell and Reid, 1965).

The second milk sample collected was used for FA analysis essentially as described by Sukhija and Palmquist (1988). Prior to analysis, the samples were thawed, shaken, and composited into 1 sample per cow per subperiod. For trial 1, the composited samples ($\approx 200 \mu\text{L}$) were lyophilized, resuspended in 1 mL of benzene containing C19:0 as an internal standard, and methylated using BF₃-methanol. For trial 2, composited samples were lyophilized, resuspended in 1 mL of hexane containing C13:0 as an internal standard, and methylated using BF₃-methanol. The resulting FA methyl esters were extracted in hexane and injected onto a Supelco Inc. (Bellefonte, PA) SP-2560 capillary GC column (100 m $\times$ 0.25 mm $\times$ 0.2 μm) for FA profile analysis, with a run time of 67 min. The temperature gradient used to quantify FA in trial 1 did not detect C4:0, which typically comprises about 5% FA in milk (Jensen, 2002). Proportions of FA are reported as a per-

centage (g/100 g) of total FA detected in each analysis. Yields of individual FA were calculated as milk fat yield $\times$ 0.933 g of total FA/g of fat (Glasser et al., 2007) $\times$ proportion of FA (g of FA/g of total FA).

Feed and Orts samples were collected during each dietary period. All samples were dried in a 55°C forced-air oven for 72 h to determine DM concentration used for calculation of DMI. Feed samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA), then composited by period. Feed samples were analyzed for DM (overnight at 105°C), ash (8 h at 450°C), NDF, CP, ether extract, total sugars, and starch. The concentration of NDF was determined (Van Soest et al., 1991) in the presence of sodium sulfite and amylase with an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). The CP concentration was determined by oxidation and detection of N₂ (Leco Analyzer; Leco Corp., St. Joseph, MI). The crude fat concentration was determined by ether extraction (AOAC, 2000: method 920.9). Total sugars were quantified for TMR samples by incubation with invertase, followed by measurement of reducing sugars (Lane and Eynon, 1923). Starch was determined by α -amylase and glucoamylase digestion (Herrera-Saldana and Huber, 1989), followed by colorimetric glucose quantification (Raabo and Terkildsen, 1960) with a commercial kit (Autokit Glucose; Wako Chemicals USA, Richmond, VA).

Ruminal contents were thawed in a cool environment and then composited by cow within period. Once composited, samples were diluted 1:1 with distilled, deionized water and mixed in a high-speed blender for 2 min. Approximately 5 mL of the blended sample was collected and placed on ice until analysis of ruminal microbial populations. Total DNA was isolated using a commercial kit (ZR Fecal DNA kit; Zymo Research Corp., Orange, CA; Stauffer et al., 2008). Isolated DNA was quantified by spectroscopy (Nanodrop-1000; NanoDrop Technologies Inc., Wilmington, DE), and sample volumes were adjusted to achieve uniform DNA concentrations across all samples. Quantitative real-time PCR was used to determine total abundance of bacterial populations using previously validated primers (forward: CCGCAACGAGCGCAACCC, reverse: CCATTGTAGCACGTGTGTAGCC) for genes encoding 16S ribosomal RNA (Denman and McSweeney, 2006). Protozoa were quantified using primers (forward: GCTTTCGWTGGTAGTGTATT, reverse: CTTGCCCTCYAATCGTWCT) designed for regions of the 18S ribosomal RNA gene and internal transcribed spacer regions (Sylvester et al., 2004). The cycle-to-threshold (CT) value obtained for total bacteria was used as the reference value for analysis of protozoal populations, and a common fluorescence

threshold was used for both assays. Efficiencies of PCR reactions determined by sample dilution were 1.79 for protozoa and 1.82 for bacteria. For each sample, the abundance of protozoa relative to total bacteria was calculated ($2^{-\Delta CT}$).

Ruminal fluid was thawed and mixed before analysis for VFA and NH₃. A composited ruminal fluid sample was taken to yield an 8-mL sample, and 2 mL of 25% (wt/vol) metaphosphoric acid was added. Composited samples were frozen, thawed, and centrifuged at 30,000 $\times g$ for 20 min, and the supernatant was collected for analysis of VFA and NH₃. Ammonia was determined colorimetrically using an autoanalyzer (Technicon Analyzer II; Technicon Industrial Systems, Buffalo Grove, IL). Ruminal VFA were measured using GLC (model 5890; Hewlett-Packard, Avondale, PA) with a flame ionization detector. The chromatograph was fitted with a 2 m $\times$ 4 mm inside diameter glass column packed with GP 10% SP-1200/1% H₃PO₄ (Supelco #1-1965; Supelco Inc.). The column was maintained at 130°C, the detector and injector were maintained at 250°C, and carrier gas (N₂) flow was 80 mL/min. The run time was approximately 5 min.

Statistical Analysis

Trial 1. Data from 35 cow periods were analyzed using mixed models including the fixed effect of treatment and the random effects of period and cow. One cow was removed from the study during period 3 because of mastitis. Linear and quadratic contrasts were used to assess the effects of molasses inclusion rate for each variable. Significance was declared at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Trial 2. Milk sample data was not collected during the first sub-plot period in the first period, and 1 cow was removed from the trial after completion of the first main-plot period. Thus, 31 total observations were used for statistical analysis. Data were analyzed using mixed models for a split-plot design including the fixed effects of diet, infusion, and their interaction, as well as the random effects of period and cow. Ruminal measures were collected only at the end of the dietary periods, so post-ruminal infusion effects and interactions were not tested for these variables. Protozoal population data were log-transformed for statistical analysis and reported means are back-transformed (Bland and Altman, 1996). Significance was declared at $P < 0.05$, and tendencies were declared at $P < 0.10$.

RESULTS AND DISCUSSION

Productivity and Milk Fat Yield

Molasses was used in a high-concentrate diet to replace a portion of corn grain as a potential approach

Table 3. Effects of molasses inclusion rate on productivity of lactating dairy cows (trial 1)

Item	Dietary molasses, DM basis				<i>P</i> -value ¹	
	0%	2.5%	5%	SEM	Lin	Quad
DMI, kg/d	26.0	26.2	25.8	1.0	0.82	0.69
Milk yield, kg/d	37.6	36.9	35.5	3.0	0.09	0.80
Milk fat, %	2.61	2.65	3.01	0.21	<0.01	0.09
Milk protein, %	3.35	3.32	3.31	0.09	0.25	0.88
Milk lactose, %	4.74	4.68	4.70	0.12	0.31	0.34
Milk fat, kg/d	0.98	0.97	1.05	0.10	0.15	0.39
Milk protein, kg/d	1.25	1.21	1.16	0.08	0.03	0.91
Milk lactose, kg/d	1.80	1.75	1.69	0.17	0.11	0.95
MUN, mg/dL	12.5	11.7	11.6	0.7	0.04	0.44
FCM, kg/d	32.0	31.6	32.2	2.7	0.86	0.64
SCM, kg/d	31.0	30.3	30.5	2.6	0.67	0.68
ECM, kg/d	34.5	33.8	33.9	2.7	0.65	0.73
ECM/DMI	1.33	1.28	1.32	0.08	0.78	0.32

¹Contrasts: Lin = linear effect of molasses inclusion rate; quad = quadratic effect of molasses inclusion rate.

to help alleviate MFD. Increasing the inclusion rate of molasses tended to linearly decrease milk yield ($P = 0.09$; Table 3) in trial 1. However, molasses increased milk fat concentration (linear effect: $P < 0.001$, quadratic effect: $P = 0.09$), resulting in similar yields of FCM and SCM across treatments (Table 3). Despite the highly significant effect of molasses on milk fat concentration, milk fat yield was not significantly altered by treatment. Molasses inclusion had no effect on DMI or feed efficiency measured as ECM/DMI.

In trial 2, molasses had no effect on DMI (Table 4), and molasses increased milk fat concentration ($P < 0.01$) with no significant effect on milk yield, in contrast to the negative effect of dietary molasses on milk yield in trial 1. Molasses did not significantly alter yields of

milk fat ($P = 0.25$) or ECM ($P = 0.18$). During trial 2, molasses had no significant effects on the content or yield of protein, lactose, or MUN concentrations. With the information from both trial 1 and 2, replacing corn grains with 5% molasses (DM basis) during MFD appears to increase milk fat content by approximately 10% but increases milk fat yield by a more modest 5%.

In one of the few previous studies in which a sugar source was tested in an MFD environment, Kellogg (1969) partially replaced ground milo with sucrose. Neither milk yield nor fat content was linearly increased in response to 5, 10, or 15% dietary sucrose, leading Kellogg (1969) to conclude that sucrose was unable to alleviate MFD induced by a high-concentrate diet. However, if only the 5% sucrose treatment in that

Table 4. Effects of treatment on productivity of lactating dairy cows (trial 2)

Item	Dietary molasses, DM basis				SEM	<i>P</i> -value ¹	
	Water		AA			Molasses	AA
	0%	5%	0%	5%			
DMI, kg/d	24.5	24.4	23.9	24.6	1.3	0.41	0.66
Milk yield, kg/d	28.3	28.6	29.0	28.1	2.8	0.70	0.86
Milk fat, %	2.68	2.90	2.75	2.98	0.21	0.01	0.23
Milk protein, %	3.45	3.37	3.44	3.44	0.13	0.41	0.37
Milk lactose, %	4.91	4.92	4.93	4.90	0.08	0.74	0.99
Milk fat, kg/d	0.79	0.83	0.81	0.85	0.11	0.25	0.49
Milk protein, kg/d	0.97	0.95	0.98	0.96	0.09	0.43	0.73
Milk lactose, kg/d	1.41	1.42	1.42	1.40	0.14	0.81	0.89
MUN, mg/dL	11.6	11.2	11.4	11.3	0.7	0.30	0.97
FCM, kg/d	24.9	25.8	25.7	25.7	2.9	0.51	0.57
SCM, kg/d	24.2	24.9	25.0	24.8	2.6	0.67	0.53
ECM, kg/d	26.6	27.3	27.5	27.3	2.8	0.18	0.45
ECM/DMI	1.04	1.07	1.05	1.07	0.094	0.67	0.67
BW gain, ² kg/28 d	15.9	−1.3	—	—	7.0	0.07	—

¹Contrasts: molasses = effect of dietary molasses; AA = effect of AA infusion. No molasses $\times$ AA interactions were significant for reported variables ($P > 0.15$).

²BW were measured at the end of dietary treatment periods only.

study is considered, the results are similar to what was observed in the current study; milk fat yield numerically increased by 10% compared with the 0% sucrose treatment (Kellogg, 1969). Therefore, responses to molasses observed in the current study (which cannot be attributed exclusively to the sugar fraction of molasses) are not inconsistent with past responses to moderate addition of dietary sugar.

Milk Protein

Replacement of corn grain with molasses caused a decrease in MUN in trial 1 ($P = 0.04$; Table 3), perhaps reflecting the modest decreases in dietary CP content and increased microbial protein production as molasses was included in diets. Increasing dietary molasses linearly decreased milk protein yield in trial 1 ($P = 0.03$; Table 3), with the high-molasses treatment causing a 7% decrease in protein yield, suggesting that MP supply may have limited milk protein synthesis in molasses treatments. The results in trial 1 led to the use of post-ruminal infusions of essential AA in trial 2 to test whether an increased AA supply would eliminate any negative effects of molasses on milk protein. However, in trial 2, no effects of molasses on milk protein content or yield were observed (Table 4; $P > 0.40$). Amino acid infusions did not increase milk protein content or yield, and no interaction with diet was observed (all $P > 0.35$).

In addition to the lack of an effect on milk protein, infusion of AA had no significant effect on any other endpoints measured and no interactions with diet were observed ($P > 0.10$). The NRC (2001) suggests that methionine is most limiting when diets depend upon soy or animal proteins for RUP supply. However, in diets containing high levels of DGS, lysine becomes more limiting than methionine (Schingoethe et al., 2009). When both methionine and lysine were provided in trial 2, as well as the likely third-limiting AA histidine, no response was observed. This lack of response to AA suggested that MP supply was not likely the primary limitation to milk production for either diet used in trial 2. It is possible that the greater milk production in trial 1 compared with trial 2 could have led to greater limitations in MP supply in trial 1 than in trial 2, thereby leading to the discrepant responses in milk protein in response to molasses replacing corn grain.

FA Profiles

To further understand effects of molasses on milk fat response, the FA profile of milk was measured. During trial 1, addition of molasses linearly decreased the proportion of unsaturated FA in milk ($P = 0.01$; Table

5). Molasses did not significantly alter yields of C16 or long-chain FA, but linearly increased the yield of short- and medium-chain FA ($P < 0.01$; Table 5). However, this differential response was not observed in trial 2; diet had no significant effects on total yields of de novo synthesized (short- and medium-chain) or pre-formed (long-chain) FA (Table 6; $P > 0.21$).

According to the biohydrogenation theory of MFD, individual FA have been implicated in MFD. Lock et al. (2007) found that the ability of specific *trans*-FA to directly cause MFD is questionable; however, they are almost always elevated in cases of MFD. As a result, some are used as markers of ruminal conditions that promote MFD. Under the normal pathway for biohydrogenation, linoleic acid is isomerized to *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and reduced to *trans*-11 C18:1 (Palmquist et al., 2005), but when the rumen environment is altered by concentrate feeding, an alternative pathway for biohydrogenation occurs, resulting in production of *trans*-10, *cis*-12 CLA and *trans*-10 C18:1. When molasses was added to the TMR in trial 1, a tendency existed for a linear decrease in the proportion of *trans*-10 C18:1 and total *trans*-C18:1 in milk FA ($P = 0.06$ for both). Yields of these FA linearly decreased with added molasses (both $P < 0.05$). Consistent with trial 1 findings, molasses decreased the yield of *trans*-10 C18:1 and increased the yield of *trans*-11 C18:1 in milk ($P < 0.05$; Table 6) during trial 2. The concentrations and yields of *trans*-10, *cis*-12 CLA, thought to be responsible for many cases of MFD (Bauman et al., 2008), were not significantly altered by the addition of molasses in either trial. The shifts in FA profile suggest that dietary molasses had a positive influence on biohydrogenation by promoting the normal biohydrogenation pathway and decreasing flux through the alternative pathway.

Molasses decreased the Δ^9 -desaturase index (quantified as C14:1/C14, $P < 0.01$; Tables 5 and 6) in both trials, which suggests that decreased desaturase activity in the mammary gland contributed to the decreased proportion of unsaturated FA in milk with molasses treatments. Milk fat depression is typically associated with decreased Δ^9 -desaturase activity, and decoupled effects on milk fat yield and Δ^9 -desaturase activity suggest that molasses may increase the production of a FA that inhibits Δ^9 -desaturase activity without greatly decreasing milk fat synthesis pathways in the mammary gland, as several CLA isomers have been shown to do (Harvatine et al., 2009).

Ruminal Metabolism and Microbial Populations

One key addition to trial 2 was the ability to collect ruminal digesta, allowing for assessment of changes in

Table 5. Effects of molasses inclusion rate on milk fatty acid profile and yield (trial 1)

Fatty acid	Dietary molasses, DM basis				<i>P</i> -value ¹	
	0%	2.5%	5%	SEM	Lin	Quad
Profile, g/100 g of fatty acids						
C14:0	10.7	11.0	11.8	0.6	0.01	0.41
C14:1	2.01	1.80	1.69	0.21	0.06	0.74
Short- and medium-chain (C4–C15)	25.6	26.1	28.5	1.6	0.01	0.34
C16	27.0	26.3	26.5	0.5	0.10	0.09
<i>trans</i> -10 C18:1	3.96	3.30	2.76	0.90	0.06	0.92
<i>trans</i> -11 C18:1	1.43	1.67	1.63	0.17	0.26	0.38
Total <i>trans</i> C18:1 ²	5.84	5.25	4.81	0.82	0.06	0.87
<i>cis</i> -9, <i>trans</i> -11 CLA ³	1.00	1.06	1.00	0.10	0.95	0.49
<i>trans</i> -10, <i>cis</i> -12 CLA	0.028	0.031	0.016	0.010	0.46	0.54
Total CLA	1.20	1.26	1.16	0.10	0.73	0.34
Long-chain (>C16)	47.4	47.7	44.9	1.7	0.04	0.17
Total unsaturated	43.3	42.5	39.4	2.1	0.01	0.24
Total polyunsaturated	8.40	8.50	7.88	0.42	0.06	0.13
Yield, g/d						
C14:0	101	102.8	118.6	14.9	0.01	0.24
C14:1	17.0	15.2	15.3	1.49	0.12	0.33
Short- and medium-chain (C4–C15)	244	244	290	39.2	0.01	0.17
C16	248	241	259	24.3	0.31	0.18
<i>trans</i> -10 C18:1	30.6	25.4	21.1	5.78	0.02	0.88
<i>trans</i> -11 C18:1	13.5	16.1	16.2	2.33	0.20	0.52
Total <i>trans</i> C18:1 ²	48.1	45.7	41.2	4.66	0.04	0.70
<i>cis</i> -9, <i>trans</i> -11 CLA	9.8	10.8	10.3	1.5	0.72	0.52
<i>trans</i> -10, <i>cis</i> -12 CLA	0.21	0.24	0.17	0.08	0.79	0.65
Total CLA	10.8	11.8	11.2	1.40	0.74	0.43
Long-chain (>C16)	420	427	423	27.9	0.91	0.78
Total unsaturated	379	376	368	24.26	0.52	0.86
Total polyunsaturated	74.1	76.3	74.9	6.81	0.85	0.65
Desaturase index (C14:1/C14)	0.16	0.14	0.13	0.02	<0.01	0.97

¹Contrasts: lin = linear effect of molasses inclusion rate; quad = quadratic effect of molasses inclusion rate.

²Includes *trans*-9, *trans*-10, and *trans*-11 C18:1.

³CLA = conjugated linoleic acid.

ruminal fermentation when a portion of corn grain was replaced with molasses. Dietary molasses significantly increased ruminal pH and decreased total VFA concentration in the rumen (Table 7; $P < 0.02$). Molasses also increased the molar proportions of acetate and butyrate and decreased the proportion of propionate in ruminal fluid, although these effects were not dramatic (Table 7). This finding is consistent with past reports (Heldt et al., 1999; Ribeiro et al., 2005) that sugars increase the ruminal concentration of butyrate and not propionate. In addition, adding molasses in place of corn grain decreased the abundance of protozoa relative to total bacteria (Table 7; $P < 0.02$).

Several microorganisms play key roles in biohydrogenation, and changes in ruminal microflora can potentially affect both intermediates and end products of biohydrogenation. The role that ciliated protozoa play in the rumen has been debated for many years. It has been reported that approximately three-quarters of microbial FA in the rumen are found in protozoa (Keeney, 1970), and protozoa could be an important source of polyunsaturated FA, CLA, and *trans*-11 C18:1 (Harfoot and Hazlewood, 1997). Devillard and Wallace (2006)

revisited the role of protozoa in biohydrogenation and concluded that although protozoa are rich in CLA and *trans*-11 C18:1, they do not appear to synthesize these FA from either linoleic or stearic acids. Karnati et al. (2009) investigated the role of protozoa during biohydrogenation in continuous culture fermenters subjected to unsaturated fat, monensin, or bromoethanesulfonate treatments. They reported increased flow of total C18 FA in defaunated fermenters, likely due to increased *de novo* synthesis of FA by the increased bacterial biomass and not the absence of protozoa per se. Nevertheless, it is possible that protozoa slow FA biohydrogenation by maintaining an intracellular pool of FA which are unavailable to biohydrogenating bacteria. The literature is inconsistent regarding whether dietary sugar promotes or inhibits growth of protozoal populations, and these inconsistencies are likely because of the heterogeneous nature of ruminal protozoa (Oelker et al., 2009). For example, it is possible that the dominant protozoa present in the rumen of cattle fed a high-grain diet do not respond well to the addition of sugar, whereas in a more forage-based diet, the dominant protozoa may proliferate in response to additional sugar.

Table 6. Effects of treatment on milk fatty acid profile and yield (trial 2)

Fatty acid	Dietary molasses, DM basis				SEM	<i>P</i> -value ¹	
	Water		AA			Molasses	AA
	0%	5%	0%	5%			
Profile, g/100 g of fatty acid							
C14:0	10.6	11.0	10.4	10.8	0.52	0.19	0.16
C14:1	1.50	1.31	1.39	1.31	0.22	0.04	0.14
Short- and medium-chain (C4–C15)	21.6	23.1	21.6	22.2	1.36	0.15	0.25
C16	28.0	27.8	27.7	27.4	1.18	0.55	0.10
<i>trans</i> -10 C18:1	3.30	1.81	2.82	1.75	0.70	0.01	0.27
<i>trans</i> -11 C18:1	1.58	2.15	1.76	2.11	0.26	<0.01	0.43
Total <i>trans</i> C18:1 ²	5.47	4.49	5.17	4.31	0.57	<0.05	0.39
<i>cis</i> -9, <i>trans</i> -11 CLA ³	0.91	1.15	1.00	1.10	0.14	<0.01	0.54
<i>trans</i> -10, <i>cis</i> -12 CLA	0.035	0.030	0.035	0.025	0.006	0.06	0.47
Total CLA	1.10	1.35	1.20	1.29	0.14	0.02	0.68
Long-chain (>C16)	50.4	49.1	50.7	50.3	1.20	0.27	0.15
Total unsaturated	42.7	40.4	42.4	41.0	2.07	<0.01	0.61
Total polyunsaturated	7.39	7.74	7.44	7.67	0.37	0.01	0.89
Yield, g/d							
C14:0	82.2	85.9	81.7	86.9	14.2	0.90	0.75
C14:1	9.77	9.52	9.39	9.39	1.14	0.72	0.18
Short- and medium-chain (C4–C15)	170	182	172	181	31	0.21	0.89
C16	201	211	202	214	25	0.25	0.71
<i>trans</i> -10 C18:1	17.9	11.6	14.8	11.8	3.29	0.03	0.08
<i>trans</i> -11 C18:1	13.7	16.7	14.8	17.5	3.61	0.05	0.38
Total <i>trans</i> C18:1 ²	34.8	32.5	33.5	33.1	3.29	0.50	0.84
<i>cis</i> -9, <i>trans</i> -11 CLA	6.52	7.67	6.69	7.10	1.45	0.10	0.56
<i>trans</i> -10, <i>cis</i> -12 CLA	0.21	0.20	0.21	0.19	0.03	0.43	0.53
Total CLA	8.56	10.01	9.45	10.08	1.84	0.08	0.22
Long-chain (>C16)	368	376	376	398	53	0.46	0.31
Total unsaturated	302	303	305	318	36	0.61	0.32
Total polyunsaturated	54.9	59.1	55.6	61.4	8.3	0.11	0.33
Desaturase index (C14:1/C14)	0.13	0.11	0.12	0.11	0.02	<0.01	0.24

¹Contrasts: molasses = effect of dietary molasses; AA = effect of AA infusion. No molasses × AA interactions were significant for reported variables ($P > 0.05$).

²Includes *trans*-9, *trans*-10, and *trans*-11 C18:1.

³CLA = conjugated linoleic acid.

Although molasses treatments had multiple effects on nutrient densities (i.e., decreased starch and increased ash contents), the observed effects of molasses on measures of ruminal metabolism are consistent with reported responses to dietary sucrose. Penner and Oba (2009) fed early-lactation cows diets including 0 or 4.7%

sucrose and observed that sucrose tended to increase mean ruminal pH, decreased the proportion of *trans*-C18:1 FA in milk, and tended to increase milk fat yield by 7%. Furthermore, Oelker et al. (2009) reported that ruminal pH was not decreased by addition of sugars into diets at a rate of less than 5% of DM. Given that

Table 7. Effects of molasses inclusion rate on measures of ruminal metabolism (trial 2)

Ruminal	Dietary molasses		SEM	<i>P</i> -value
	0%	5%		
pH	5.73	5.87	0.06	0.02
Total VFA, mM	140.8	132.7	4.6	<0.01
Acetate, mol/100 mol	46.3	46.9	0.9	0.04
Propionate, mol/100 mol	28.7	27.4	1.4	0.01
Butyrate, mol/100 mol	16.7	17.7	1.0	0.04
Valerate, mol/100 mol	4.9	4.9	0.3	0.78
Ammonia, mM	7.9	7.3	0.88	0.32
Protozoa, DNA copies relative to copies of bacterial DNA ¹	0.0029	0.0007	0.0021	0.02

¹Protozoal population data were natural log-transformed for statistical analysis, and reported least squares means are from back-transformation. Least squares means of log-transformed values were −5.81 for 0% molasses and −7.21 for 5% molasses with an SEM of 0.97.

sugar sources are fermented very rapidly in the rumen, the observed positive effects on rumen pH are somewhat surprising. However, ruminal pH is influenced not only by acid production, but also by buffering and acid removal. One possible explanation for this response is that VFA uptake from the rumen is stimulated by molasses. Molasses generally increases butyrate production, and butyrate stimulates blood flow to the ruminal epithelium to a greater extent than other VFA (Sellers et al., 1964; Thorlacius, 1972). Increased blood flow to the epithelium would result in more rapid transfer of VFA from epithelial cells to the bloodstream, in turn creating an increased gradient between the rumen and the epithelial cells. This potential increase in uptake rate could explain the decrease in ruminal VFA concentration and increased ruminal pH in the molasses treatment. However, numerous other mechanisms could also explain the response (Penner and Oba, 2009; Firkins, 2010). Regardless of the mechanism underlying the increased ruminal pH in cows fed sugar sources, decreased acid load provides a logical explanation for the improvement in biohydrogenation. Fiber-digesting bacteria are thought to be primarily responsible for ruminal biohydrogenation of FA (Harfoot and Hazlewood, 1997), and these bacteria are especially susceptible to growth inhibition by low pH.

CONCLUSIONS

Across 2 trials in which molasses replaced corn grain at up to 5% of dietary DM, increasing molasses content of the diet significantly increased milk fat concentration. However, effects on milk yield tended to be negative, and in trial 1, milk protein yield decreased when molasses replaced corn grain. Nevertheless, these data suggest that in an MFD environment, dietary molasses increases ruminal pH, promotes maintenance of normal ruminal FA biohydrogenation, and leads to recovery of milk fat yield by 5 to 10%.

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