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

### Abstract

Molasses has long been used in animal feeds for palatability and as a binding agent to ensure uniform consumption of essential nutrients. Recent work with molasses in highly fermentable diets has revealed that molasses might offer additional benefits in dairy rations. Feeding highconcentrate diets increases the risk of milk fat depression by disrupting the normal pathway of fatty acid biohydrogenation in the rumen. Preliminary research conducted at Kansas State University and other universities has indicated that dietary sugars have the potential to increase milk fat synthesis during milk fat depression. In this study, we sought to understand the reasons for this beneficial effect of molasses on milk fat synthesis. Despite the fact that molasses provides readily fermentable sugar, replacing 5% of dietary corn grain with molasses increased ruminal pH, improved fatty acid biohydrogenation, and shifted the profile of fermentation acids in a manner suggesting that growth of fiber-digesting bacteria was improved. Results of several studies suggest that 5% dietary molasses can increase milk fat yield by 5 to 10%, and the current study indicates that this effect is driven by a stabilization of ruminal pH and biohydrogenation; Dairy Day, 2009, Kansas State University, Manhattan, KS, 2009; Dairy Research, 2009 is known as Dairy Day, 2009

### Keywords

Kansas Agricultural Experiment Station contribution; no. 10-103-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 1021; Dairy Day, 2009; Dairy; Feed intake; Milk fatty acids; Milk yield; Molasses; Rumen pH

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# Dietary Molasses Increases Ruminal pH and Enhances Ruminal Biohydrogenation During Milk Fat Depression

*C. A. Martel, E. C. Titgemeyer, and B. J. Bradford*

## Summary

Molasses has long been used in animal feeds for palatability and as a binding agent to ensure uniform consumption of essential nutrients. Recent work with molasses in highly fermentable diets has revealed that molasses might offer additional benefits in dairy rations. Feeding high-concentrate diets increases the risk of milk fat depression by disrupting the normal pathway of fatty acid biohydrogenation in the rumen. Preliminary research conducted at Kansas State University and other universities has indicated that dietary sugars have the potential to increase milk fat synthesis during milk fat depression. In this study, we sought to understand the reasons for this beneficial effect of molasses on milk fat synthesis. Despite the fact that molasses provides readily fermentable sugar, replacing 5% of dietary corn grain with molasses increased ruminal pH, improved fatty acid biohydrogenation, and shifted the profile of fermentation acids in a manner suggesting that growth of fiber-digesting bacteria was improved. Results of several studies suggest that 5% dietary molasses can increase milk fat yield by 5 to 10%, and the current study indicates that this effect is driven by a stabilization of ruminal pH and biohydrogenation.

## Introduction

Satisfactory milk fat production by lactating dairy cows has great economic value to dairy producers. Fat is the most variable component of milk, and it can be affected by many factors including diet, genetics, physiological state, and environment. Milk fat depression (**MFD**) is a decrease in milk fat yield of up to 50% with no change in milk yield or yield of other milk components. Milk fat depression occurs when dietary factors influence ruminal fermentation and biohydrogenation of unsaturated fatty acids (**FA**), resulting in the production of unique FA that decrease synthesis of milk fat in the mammary gland. Normal biohydrogenation converts linoleic acid (*cis*-9, *cis*-12 C<sub>18:2</sub>) to stearic acid (C<sub>18:0</sub>) using *cis*-9, *trans*-11 conjugated linoleic acid and *trans*-11 C<sub>18:1</sub> FA as intermediates. When MFD occurs, conversion of linoleic acid to stearic acid goes through an alternative pathway that produces *trans*-10, *cis*-12 conjugated linoleic acid, and *trans*-10 C<sub>18:1</sub> FA as intermediates, both of which are capable of inducing MFD.

Because of increasing feed costs, use of by-products like distillers grains has increased during the past few years. Although these by-products can provide less expensive dietary feedstuffs, they are imperfect. Unlike many other corn-derived feedstuffs, distillers grains contain about 10% unsaturated fat, which can impair ruminal biohydrogenation and promote MFD. As a result, few commercial dairies feed more than 10% distillers grains.

A preliminary study conducted at Kansas State University in 2008 found that inclusion of 5% molasses in a high-concentrate diet could decrease production of MFD-inducing FA. We hypothesized that molasses enhances milk fat synthesis by supporting the growth of bacteria responsible for FA biohydrogenation in the rumen, resulting in decreased absorption of FA that induce MFD. In addition to the signs of improved milk fat synthesis in molasses-fed cows, however, our previous study also showed a decrease in milk protein yield with the 5% molasses treatment. Our overall objective in this study was to evaluate the effects of adding molasses to a high-concentrate diet containing 20% distillers grains, with or without supplemental amino acids.

## Experimental Procedures

Six rumen-cannulated, multiparous, late-lactation Holstein cows ( $220 \pm 18$  days in milk) were used to evaluate effects of adding molasses, with or without supplemental amino acids, on ruminal traits and milk composition. The control diet was formulated with the intention of causing MFD and included 37% forage and 20% corn dried distillers grains with solubles, resulting in a diet with 24.5% neutral detergent fiber, 48.7% non-fiber carbohydrate, and 4.7% ether extract (Table 1). Dietary treatments were formulated such that a 5% inclusion rate of cane molasses replaced a portion of the corn grain. Dietary treatments were fed for 28 days, allowing 16 days for diet adaptation and the final 12 days for 2 abomasal infusion periods (Figure 1). In the preliminary study, increasing the inclusion rate of molasses decreased milk protein yield, which suggested that metabolizable protein supply may be inadequate, thus limiting milk protein synthesis. As a result of those findings, either water or the 3 most limiting amino acids were infused into the abomasum to test the effect of additional amino acid supply. Throughout the experiment, cows were housed in a tie-stall facility, milked thrice daily (0500, 1300, and 2100 hours), and fed twice daily (0630 and 1500 hours) for ad libitum intake.

Milk production data and samples were collected during the final 4 days of each infusion period for analysis of milk components and FA profiles (Figure 1). Use of cannulated cows facilitated ruminal digesta collection to assess measures of ruminal fermentation. Ruminal contents were collected at 9-hour intervals on days 26 to 28 of each dietary period, which represented every 3 hours of a 24-hour period. Rumen samples were analyzed for pH as well as volatile FA and ammonia concentrations.

## Results and Discussion

### *Milk Protein*

Our preliminary study showed that increasing the inclusion rate of dietary molasses linearly decreased milk protein yield. This prompted further investigations into the effects of molasses on milk protein. In the present study, essential amino acids were infused into the abomasum to test whether molasses created a metabolizable protein limitation that could be overcome with additional amino acids. Amino acid infusions had no interaction with molasses for milk protein content or yield (Table 2). In addition, molasses had no direct effect on milk protein content or yield. Therefore, these results do not support previous findings that dietary molasses negatively affects milk protein yield during MFD.

### *Productivity and Milk Fat*

Consistent with previous findings, 5% molasses had no effect on dry matter intake during MFD (Table 2). Molasses tended ( $P = 0.06$ ) to decrease body weight gain compared with the control diet ( $-7$  vs.  $+35 \pm 20$  lb during 28 days). Dietary molasses increased ( $P < 0.01$ ) milk fat concentration with no significant effect on milk yield. Neither content nor yield of protein, lactose, or urea nitrogen was changed by addition of molasses.

To further understand the role of molasses in FA biohydrogenation, milk FA analysis was conducted. Dietary molasses decreased the yield of *trans*-10 C<sub>18:1</sub> and increased ( $P < 0.01$ ) the yield of *trans*-11 C<sub>18:1</sub> in milk, key intermediates in alternative and normal biohydrogenation pathways, respectively (Table 3). Shifts in FA profiles indicated that dietary molasses had a positive influence on biohydrogenation by promoting normal biohydrogenation and decreased use of the alternative pathway.

***Ruminal Metabolism***

Analysis of ruminal fluid showed that dietary molasses increased ( $P < 0.01$ ) pH and decreased total volatile FA concentration in the rumen (Table 4). Molasses increased ( $P < 0.04$ ) molar proportions of acetate and butyrate but decreased ( $P < 0.01$ ) the proportion of propionate in ruminal fluid.

Replacing 5% dietary corn with cane molasses (on a dry matter basis) in a low-forage, high-concentrate diet increased ruminal pH, promoted the normal pathway of ruminal biohydrogenation, and decreased the production of a key MFD-inducing FA. Dietary molasses increased milk fat content in a diet used to induce MFD without changing milk component yield. Considered along with our prior results, these data indicate that molasses has the potential to mildly increase milk fat yield by promoting normal ruminal biohydrogenation and decreasing absorption of MFD-inducing FA.

**Table 1. Diet composition (% of dry matter)**

Item	Dietary molasses, %	
	0	5
Ingredient		
Corn silage	25.5	25.5
Alfalfa hay	12.6	12.6
Corn dried distillers grains with solubles	19.8	19.8
Ground corn grain	33.6	28.8
Molasses	—	4.8
Soybean meal	4.1	4.1
Expeller soybean meal	2.7	2.7
Limestone	1.1	1.1
Trace mineral salt	0.4	0.4
Micronutrient premix	0.2	0.2
Nutrient		
Dry matter, % as is	66.4	65.8
Crude protein	16.2	16.1
Neutral detergent fiber	24.5	24.5
Non-fiber carbohydrate	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

**Table 2. Effects of treatment on productivity of lactating dairy cows**

Item	Infusion				SEM	<i>P</i> value <sup>1</sup>	
	Water		Amino acids			Molasses	AA
	Dietary molasses						
	0%	5%	0%	5%			
Dry matter intake, lb/day	54.0	53.8	52.7	54.2	2.9	0.41	0.66
Milk yield, lb/day	62.4	63.1	63.9	61.9	6.2	0.70	0.86
Solids-corrected milk, lb/day	53.4	54.9	55.1	54.7	5.7	0.67	0.53
Energy-corrected milk, lb/day	58.6	60.2	60.6	60.2	6.2	0.18	0.45
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, lb/day	1.74	1.83	1.80	1.87	0.24	0.25	0.49
Milk protein, lb/day	2.14	2.09	2.16	2.12	0.20	0.43	0.73
Milk lactose, lb/day	3.11	3.13	3.13	3.08	0.31	0.81	0.89
Milk urea nitrogen, mg/dL	11.6	11.2	11.4	11.3	0.7	0.30	0.97

<sup>1</sup> Molasses = effect of dietary molasses; AA = effect of amino acid infusion. All molasses × AA effects were nonsignificant for reported variables ( $P > 0.15$ ).

**Table 3. Effects of treatment on yield of selected milk fatty acids**

Fatty acid, g/day	Infusion				SEM	<i>P</i> value <sup>1</sup>	
	Water		Amino acids			Molasses	AA
	Dietary molasses						
	0%	5%	0%	5%			
<i>trans</i> -10 C <sub>18:1</sub>	16.8	10.9	12.8	10.4	3.3	0.01	0.15
<i>trans</i> -11 C <sub>18:1</sub>	12.2	14.7	12.3	14.1	3.0	<0.01	0.76
total <i>trans</i> C <sub>18:1</sub>	32.3	28.7	27.8	27.8	3.0	0.28	0.13
<i>trans</i> -10, <i>cis</i> -12 CLA <sup>2</sup>	0.19	0.18	0.18	0.15	0.03	0.37	0.42

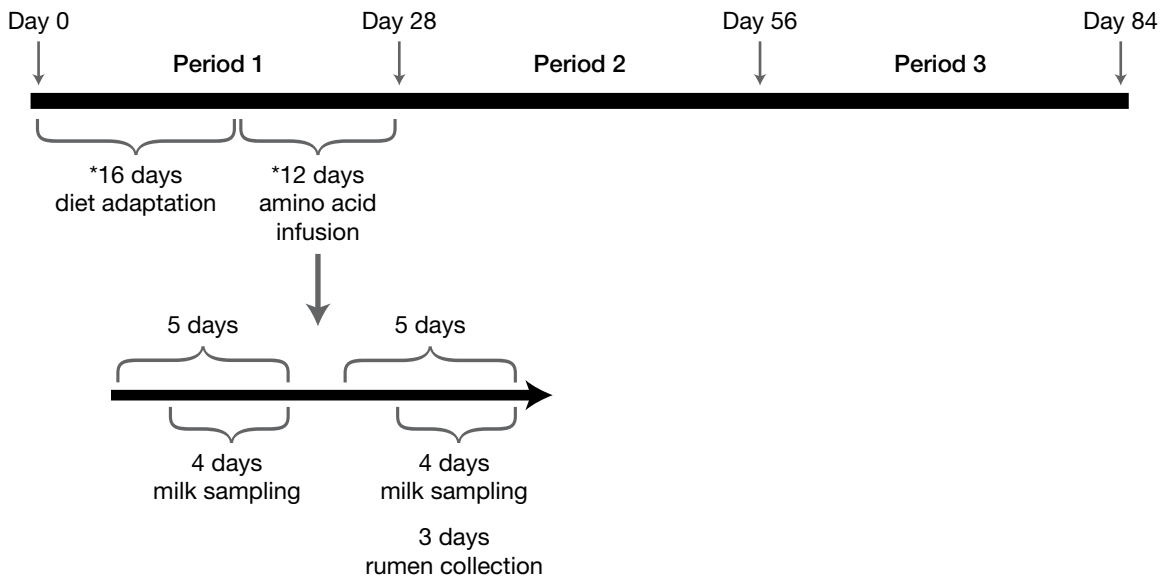
<sup>1</sup> Molasses = effect of dietary molasses; AA = effect of amino acid infusion. All molasses × AA effects were nonsignificant for reported variables ( $P > 0.15$ ).

<sup>2</sup> Conjugated linoleic acid.

**Table 4. Effects of molasses inclusion rate on measures of ruminal fermentation**

Item	Dietary molasses, %		SEM	<i>P</i> value
	0	5		
Ruminal pH	5.73	5.87	0.06	0.02
Total volatile fatty acids, 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, mg/dL	7.86	7.35	0.88	0.32

## NUTRITION AND FEEDING



**Figure 1. Experimental protocol.**

Each period included 16 days of diet adaptation and 12 days of amino acid infusion and data collection.