

Dietary Molasses Enhances Ruminal Biohydrogenation and Increases Mammary Gland *De Novo* Fatty Acid Synthesis During Milk Fat Depression

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INTRODUCTION

Production of ethanol is increasing rapidly in the United States. In the past 5 years alone, ethanol production capacity has more than doubled, as has production of distillers grains (typically with solubles added and often dried as **DDGS**). Although much work has been done to assess the effects of DDGS on productivity of lactating dairy cows, many nutritionists and dairy producers remain skeptical of its value in lactation diets. Reports of milk fat depression in herds incorporating DDGS are widespread, and some controlled studies have demonstrated that high inclusion rates can depress milk fat yield (Owen and Larson, 1991; Cyriac et al., 2005). These concerns continue to limit the utilization of DDGS in the dairy industry.

Milk fat depression (**MFD**) is caused by an interaction of dietary factors which influence ruminal fermentation and the availability of unsaturated fatty acids. Unique fatty acids produced in this rumen environment are capable of altering mammary function to decrease synthesis of milk fat. Therefore, unsaturated fatty acids provided by DDGS can lead to depressed milk fat yield.

One way to prevent milk fat depression when feeding DDGS is to increase dietary fiber content, but unfortunately, high fiber diets limit energy intake and productivity. Increasing dietary sugar content may provide an alternative method of preventing milk fat depression from DDGS. Broderick and Radloff (2004) reported that replacing high-moisture corn with molasses resulted in improved fiber digestibility; this likely reflects a stimulatory effect of molasses on fiber-digesting ruminal bacteria. Fiber-digesting bacteria are thought to be primarily responsible for ruminal biohydrogenation of fatty acids (Harfoot and Hazlewood, 1988), suggesting that dietary molasses may be capable of enhancing biohydrogenation of unsaturated fatty acids. Complete biohydrogenation of unsaturated fatty acids eliminates potential negative effects on milk fat

synthesis; therefore, molasses may be capable of preventing diet-induced milk fat depression.

In support of this hypothesis, inclusion of molasses at 4% of dietary DM in a low-fiber diet increased milk fat yield (Morales et al., 1989). Likewise, in one trial reported by Broderick and Radloff (2004), inclusion of dried molasses in a low-fiber ration had a quadratic effect on milk fat yield, with increases observed at 4 and 8% of diet DM. However, there are relatively few published studies reporting the effects of molasses in lactation diets, and none have been specifically designed to evaluate the ability of molasses to prevent diet-induced milk fat depression. Our objective was to determine if replacing corn grain with molasses at up to 5% of diet DM would prevent milk fat depression from a high concentrate ration including DDGS.

MATERIALS AND METHODS

Animals and treatments. Twelve second-lactation Holstein cows (696 ± 52 kg BW, 134 ± 37 DIM, mean \pm SD) were randomly assigned to square and sequence within square in a replicated 3 x 3 Latin square design balanced for carryover effects. The control diet was formulated with the intention of causing MFD, and included 36.6% forage and 21.2% corn DDGS, resulting in a diet with 26.2% NDF, 46.4% NFC, and 4.4% crude lipid. 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. Molasses used in this study was approximately 79 degrees Brix and 71.9% DM, and did not include any additives. Composition and nutrient densities for the experimental diets are shown in **Table 1**. A common base mix representing 95% of diet DM was prepared daily, and ground corn grain and/or molasses was added to complete each TMR. Throughout the experiment, cows were housed in a tie-stall facility, milked twice daily (500 h and 1600 h), and fed twice daily (630 h and 1700 h) for *ad libitum* intake.

Data and sample collection and analysis. Treatment periods were 28 d, with 14 d for diet adaptation and 14 d for sample and data collection. All cows were treated with Posilac on days 1 and 15 of each period. 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; one sample was used for component analysis and the other was stored at -20°C for analysis of fatty acid profile. Milk samples were analyzed to determine concentrations of fat, protein, lactose, and urea nitrogen (Heart of America DHIA, Manhattan). Milk samples used for fatty acid analysis were thawed, shaken, and composited into 1 sample per cow/period. The composited samples (~200 µL) were lyophilized, resuspended in 1 mL of benzene containing C19:0 as an internal standard, and methylated using BF₃-methanol. The resulting fatty acid methyl esters were extracted in hexane and injected onto a Supelco SP-2560 capillary GC column for fatty acid profile analysis. Butyric acid is obscured by a solvent peak in this method, so fatty acid profiles were analyzed without this milk fat component.

One cow was removed from the study early in period 3 because of mastitis. Data from the remaining 35 cow periods were analyzed using mixed models including the fixed effect of treatment and the random effects of period and cow. 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 $P < 0.10$.

RESULTS AND DISCUSSION

Feeding a high-concentrate diet including 21% corn DDGS decreased milk fat concentration from 3.28% prior to the study to 2.61% during the study. Despite the extreme nature of the diet (predicted NE_L density of 0.81 Mcal/lb DM), cows appeared healthy and ate well throughout the study. In addition, feed efficiencies values (mean: 1.33 kg ECM /kg DMI) suggest that the control diet did not dramatically impair nutrient digestion.

Productivity and milk fat yield. The effects of molasses inclusion on productivity in this setting are shown in **Table 2**. Treatments had no effect on dry matter intake or feed efficiency (measured as ECM/DMI). Increasing molasses inclusion rate tended to linearly decrease milk yield ($P = 0.09$). However, molasses increased milk fat concentration (linear effect: $P < 0.001$, quadratic effect: $P = 0.09$), resulting in similar yields of fat-corrected milk and solids-corrected

milk across treatments. Despite the highly significant effect of molasses on milk fat concentration, milk fat yield was not significantly altered by treatment.

To further investigate the effects of dietary molasses on milk fat synthesis, we measured the profile of fatty acids in milk, and a summary of this data is shown in **Table 3**. Addition of molasses decreased the proportion of numerous unsaturated fatty acids in milk, and linearly decreased the proportion of total unsaturated fatty acids ($P = 0.01$). Of particular interest, increasing dietary molasses tended to linearly decrease the proportion of *trans*-10 C18:1 and total *trans*-C18:1 fatty acids in milk ($P = 0.06$ for both). More importantly, total yields of these fatty acids were linearly decreased by molasses (both $P < 0.05$). Although the ability of specific *trans* fatty acids to directly cause milk fat depression is questionable (Lock et al., 2007), they are nearly always elevated in cases of MFD, and can be used as markers of ruminal conditions that promote MFD. In contrast, molasses inclusion did not significantly alter concentration or yield of *trans*-10, *cis*-12 CLA, the fatty acid thought to be responsible for many cases of MFD (Bauman et al., 2008). Nevertheless, the significant decrease in milk *trans* fatty acid secretion suggests that molasses inclusion enhanced ruminal fatty acid biohydrogenation.

In severe cases of MFD, both *de novo* fatty acid synthesis (responsible for short- and medium-chain fatty acids in milk) and utilization of circulating fatty acids (the source of long-chain fatty acids in milk) are decreased. However, the enzymes responsible for these two sources of fatty acids are different, and temporal responses to fatty acid infusions suggests that *de novo* synthesis and uptake of preformed fatty acids may be altered independently (Baumgard et al., 2000). In the current study, inclusion of molasses did not significantly alter yields of C16 or long-chain fatty acids, but linearly increased the yield of short- and medium-chain fatty acids ($P < 0.01$). This response indicates a specific effect of dietary molasses on *de novo* fatty acid synthesis in the mammary gland. The mechanism mediating this response is unclear, but it is possible that molasses altered ruminal biohydrogenation in a manner that decreased ruminal production of a fatty acid which specifically inhibits enzymes involved in fatty acid synthesis.

Additionally, increasing molasses inclusion rate linearly decreased the $\Delta 9$ -desaturase index (quantified as C14:1/C14:0, $P < 0.01$), which suggests that decreased desaturase activity in the mammary gland contributed to the decreased proportion of unsaturated fatty acids with molasses. MFD is typically associated with decreased $\Delta 9$ -desaturase activity, but the index suggests that activity was elevated in the control diet, and at least one past study found that diet-induced MFD was associated with increased $\Delta 9$ -desaturase indices (Bradford and Allen, 2004). In the future, this index may prove useful for segregating different causes of MFD on-farm.

Milk protein yield. Increasing dietary molasses linearly decreased milk protein yield ($P = 0.03$, Table 3), with the high molasses treatment causing a 7% decrease in protein yield. We observed a decrease in milk urea nitrogen as dietary molasses increased ($P = 0.04$), suggesting that metabolizable protein supply may have limited milk protein synthesis in the molasses treatments. Dietary crude protein was similar across diets (Table 1), and neither corn grain nor cane molasses at 5% of diet DM provided a substantial amount of the dietary protein. Therefore, treatment effects on milk protein yield are likely due to differences in ruminal metabolism. Molasses provides sugar that is more rapidly fermented than the starch in the corn grain that it replaced. This increase in rapidly-available carbohydrate, coupled with a relatively low concentration of rumen-degradable protein in these diets (~60% of CP, 2001 NRC), may have decreased microbial protein synthesis because of carbohydrate/protein asynchrony. Milk urea nitrogen is highly correlated with ruminal ammonia concentrations, and milk urea nitrogen concentrations were relatively low in this study, suggesting that ruminal ammonia may have been limiting for the molasses treatments. In support of this interpretation, Sannes et al. (2002) found that 3% dietary sucrose decreased microbial protein synthesis and milk protein yield in a diet with protein composition similar to the one in this study. In contrast, Broderick and Radloff (2004) reported increased urinary purine derivative excretion and milk protein yield with molasses supplementation in rations containing substantial non-protein nitrogen.

CONCLUSIONS

Replacing up to 5% of dietary corn with cane molasses linearly increased the yield of short- and medium-chain fatty acids in milk, indicating a positive effect on *de novo* fatty acid synthesis in a milk fat depression environment. However, molasses tended to linearly decrease milk yield and linearly decreased milk protein yield, resulting in no net effect on energy- or solids-corrected milk yield. These results suggest that there is potential for sources of dietary sugar to prevent milk fat depression, but further research is needed to determine when sugar sources might be most effective.

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Table 1. Ingredient and Nutrient Composition of Diets¹

	Treatment ²		
	0%	2.5%	5%
Ingredient			
Corn silage	24.7	24.7	24.7
Alfalfa hay	11.9	11.9	11.9
Corn DDGS	21.2	21.2	21.2
Ground corn grain	33.9	31.4	28.9
Molasses	-	2.5	5.0
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	0.4	0.4	0.4
Micronutrient premixes	0.2	0.2	0.2
Nutrient			
Dry Matter	64.3	64.1	63.9
Crude protein	17.4	17.2	17.1
Neutral detergent fiber	26.2	26.3	26.3
Non-fiber carbohydrate	46.4	46.3	46.2
Ether extract	4.4	4.4	4.3
Ash	5.5	5.7	5.9

¹ Values other than dry matter are expressed as a percentage of diet dry matter.

² Treatment designations indicate the inclusion rate of molasses (DM basis).

Table 2. Effects of Molasses Inclusion Rate on Productivity of Lactating Dairy Cows

	Treatment ¹			SEM	P value ²	
	0%	2.5%	5%		Lin	Quad
Dry matter intake, 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	0.001	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
Milk urea N, 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/DMI	1.33	1.28	1.32	0.08	0.78	0.32

¹ Treatment designations indicate the inclusion rate of molasses (DM basis).

² Contrasts: Lin = Linear effect of molasses inclusion rate; Quad = quadratic effect of molasses inclusion rate.

Table 3. Effects of Molasses Inclusion Rate on Milk Fatty Acid Profile and Yield

	Treatment ¹			SEM	P value ²	
	0%	2.5%	5%		Lin	Quad
g/100 g 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
<i>trans</i> -10 C18:1	3.96	3.30	2.76	0.90	0.06	0.92
Total <i>trans</i> C18:1 ³	5.84	5.25	4.81	0.82	0.06	0.87
<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
Total polyunsaturated	8.40	8.50	7.88	0.42	0.06	0.13
Total unsaturated	43.3	42.5	39.4	2.1	0.01	0.24
Short- and medium-chain (< C16)	25.6	26.1	28.5	0.02	0.01	0.34
Long-chain (> C16)	47.4	47.7	44.9	1.7	0.04	0.17
g/d						
<i>trans</i> -10 C18:1	32.9	27.2	22.6	6.2	0.02	0.88
Total <i>trans</i> C18:1 ³	51.5	49.0	44.1	5.0	0.04	0.70
Total unsaturated	406	403	394	26	0.52	0.86
Short- and medium-chain (< C16)	261	262	311	42	0.01	0.17
C16	266	258	278	26	0.31	0.18
Long-chain (> C16)	450	458	453	30	0.91	0.78

¹ Treatment designations indicate the inclusion rate of molasses (DM basis).

² Contrasts: Lin = Linear effect of molasses inclusion rate; Quad = quadratic effect of molasses inclusion rate.

³ Includes *trans*-9, *trans*-10, and *trans*-11 C18:1.