Liquid Molasses Decreases Production Linearly and Changes Enterolactone Concentrations as a Corn Meal Substitute in Organic Dairy Cows Fed Flaxseed Meal

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Abstract

The current study aimed to investigate the effects of replacing corn meal with incremental amounts of liquid molasses on production and milk concentration of enterolactone—a potent antioxidant derived from flaxseed—in organic dairy cows fed flaxseed meal-based diets. Sixteen multiparous Jersey cows were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 14 days for diet adaptation and 7 days for data and sample collection (4 periods of 21 days each). All 4 experimental diets were fed as total mixed rations and consisted (dry matter basis) of 52% grass-legume baleage, 8% grass hay, 8.5% soy hulls, 2.5% roasted soybean, and 15% flaxseed meal. Corn meal was replaced by increasing amounts of liquid molasses at 0, 4, 8, or 12% of diet dry matter. Data were analyzed using the MIXED procedure of SAS using linear, quadratic, and cubic contrasts. Dry matter intake and yields of milk and milk components decreased linearly with replacing corn meal with incremental amounts of liquid molasses, while the milk concentration of enterolactone responded cubically. The concentration of milk fat and milk true protein did not differ across treatments, whereas that of lactose decreased linearly in cows fed liquid molasses at expense of corn meal. Overall, replacing corn meal with liquid molasses negatively impacted intake and yields of milk and milk components. Further research is needed to better understand the interactions of corn meal and liquid molasses modulating the concentration of milk enterolactone in dairy cows fed flaxseed meal-based diets.

Introduction

There is a growing interest in the use of sugarcane molasses in both conventional (Oelker et al. 2009; Martel et al. 2011; Siverson et al. 2014) and organic (Soder et al. 2012; Brito et al. 2015) dairy systems in the United States. A case study conducted by Soder et al. (2012) showed that organic dairy farmers in the northeastern United States are feeding liquid molasses as the sole energy supplemental source to forage-based diets with inconsistent results in animal production and health. Because of these inconsistent results and lack of data regarding the effect of high dietary levels of liquid molasses as the major energy supplemental source on milk production and nutrient utilization in dairy cows, further research was conducted to fill these knowledge gaps.

Flaxseed meal is the richest source of the polyphenolic compound known as secoisolariciresinol diglucoside (**SDG**), which is converted in the rumen into the mammalian lignan enterolactone (Thompson et al. 1991; Côrtes et al. 2008; Gagnon et al. 2009; Zhou et al. 2009). There is a growing interest in promoting the inclusion of SDG-rich foods in human diets due to the potential human health benefits of enterolactone, including prevention of cardiovascular diseases, hypercholesterolemia, breast and prostate cancers, menopausal symptoms, and osteoporosis (Murkies et al. 1998; Adlercreutz 2002). Brito et al. (2015) reported that organic dairy cows fed liquid molasses and flaxseed meal had greater concentration

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of milk enterolactone than those fed corn meal and flaxseed meal showing that liquid molasses may select for ruminal microorganisms with greater capacity to convert feed SDG to enterolactone compared with corn meal. However, it is unknown the amount of liquid molasses that could maximize the concentration of enterolactone in milk or if there is an interaction of liquid molasses and corn meal to modulate the production of enterolactone in the rumen of lactating dairy cows and consequent appearance in milk.

Objectives

The research objective was to investigate the effects of replacing corn meal with incremental amounts of liquid molasses on production and milk composition, including enterolactone, in organic Jersey cows fed flaxseed meal-based diets.

Materials and Methods

The 84-day long study was carried out at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm (43°10'N, 70°99'W) from December, 2014 to February, 2015. Care and handling of the animals used in the study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee. Sixteen multiparous organic Jersey cows averaging (mean ± standard deviation) 99 ± 41 days in milk and 462 ± 38 kg of body weight at the beginning of the study were used. Cows were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design. Treatment sequences within each Latin square were balanced for carryover effects in subsequent periods. Each experimental period lasted 21 days with 14 days for diet adaptation and 7 days for sample and data collection.

Animals were housed in a bedded-pack barn with free access to a roof-covered feeding station equipped with a Calan doors system (America Calan Inc., Northwood, NH) located at the end of the outdoor lot. The ingredient and nutrient composition of the experimental diets are presented in Table 1. Diets were formulated to yield a 60:40 forage to concentrate ratio and were fed as total mixed rations (**TMR**). All 4 TMR consisted (dry matter basis) of 52% grass-legume baleage, 8% grass hay, 8.5% soyhulls, 2.5% roasted soybean, and 15% flaxseed meal. Corn meal was replaced by increasing amounts of liquid molasses at 0, 4, 8, or 12% of diet dry matter. Cows were fed twice a day at approximately 0700 and 1600 h and had free access to water throughout the study. The amount of TMR offered to cows was adjusted daily allowing refusals of 5 to 10% intake. Refusals were collected daily before the afternoon feeding and weighed. Feed intake was calculated by subtracting the amount of TMR offered daily from the amount of refusals. The Calan doors system was used to individualize the dietary treatments offered to cows. Samples of TMR, baleage, concentrate, and refusals were collected daily during the sampling period. All feed samples (except liquid molasses) were dried in a forced-air oven (55°C, 48 h), ground to pass through a 1-mm screen, and shipped to a commercial laboratory (Dairy One Cooperative Inc., Ithaca, NY) for nutrient analyses.

Cows were milked twice a day at 0500 and 1530 h with milk yield recorded at each milking throughout the experiment. Milk samples were collected for 4 consecutive milkings (days 20 and 21) in each sampling period, preserved in tubes containing 2-bromo-2-nitropropan-1,3 diol, pooled by cow according to morning and evening milk weights, and refrigerated at 4°C until shipped to Dairy One Cooperative Inc. for determination of fat, true protein, lactose, and milk urea-N by mid-infrared reflectance spectroscopy. Milk samples without preservative were collected concurrently, pooled, and stored at -20°C until analyzed for milk enterolactone. Milk enterolactone was hydrolyzed and extracted according to the procedures described by Gagnon et al. (2009), and analyzed colorimetrically using a commercial enzymatic immunoassay (assay kit no. 500520; Cayman Chemical Co., Ann Arbor, MI). Fecal and urine samples were collected once daily for 3 consecutive days (19 to 21) at 0700, 1200, and 1500 h. Fecal samples were collected by stimulating defecation or directly from the rectum. Spot urine samples were collected concurrently with fecal samples by stimulation of the pudendal nerve massaging the area bellow the vulva. Data were analyzed using the MIXED procedure of SAS (SAS version 9.4; SAS Inst. Inc., Cary, NC) according to a replicated 4 × 4 Latin square design. Orthogonal polynomial contrasts were used to test responses (linear, quadratic, and cubic) from incremental dietary levels of liquid molasses. All reported values are least squares means and standard error of the mean. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. The milk concentration of enterolactone was transformed (natural log) for statistical analyses, but results presented in Figure 1 were reported as adjusted mean values on the original scale of measurements.

Results and Discussion

Dry matter intake decreased linearly and averaged 19.3, 18.5, 17.8, and 17.3 kg/d in cows fed 0, 4, 8, or 12% of liquid molasses, respectively (Table 2). Milk yield also decreased linearly and averaged 18.9, 18.0, 17.8 and 16.8 kg/d in cows fed 0, 4, 8, or 12% liquid molasses, respectively (Table 2). This linear decrease in milk production is entirely explained by the linear decrease in dry matter intake. The concentrations of milk fat (mean = 5.29%) and protein (mean = 4.01%) did not differ significantly across treatments, whereas those of lactose (from 4.66 to 4.58%) and milk urea-N (from 16.8 to 16.0 mg/dL) decreased linearly with replacing corn meal with incremental amounts of liquid molasses (Table 2).

Yields of milk fat, protein, and lactose in cows fed liquid molasses at expense of corn meal, which are explained by the linear reduction in milk yield. Soder et al. (2012) reported that organic dairy farmers in the northeastern United States are feeding liquid molasses as the sole energy supplemental source to forage-based diets in levels ranging from 1.1 to 2.4 kg/cow per day (dry matter basis) with inconsistent results in animal production and health. Using the mean dry matter intake of 18 kg/d obtained from a comprehensive survey of Wisconsin organic dairies (Hardie et al. 2014) and a concentration of 41% of total sugars in liquid molasses (value derived from the current study), the 1.1- to 2.4-kg/d range reported by Soder et al. (2012) would be equivalent to 2.6 to 5.5% of added sugars from liquid molasses, with the greatest level exceeding the recommendation of 2.5 to 5.0% of added sugars in dairy diets (Firkins 2010). In the present study, the amount of added sugars ranged from 1.65 to 4.95% and was closed to the maximum recommend for dairy diets.

Thus, the negative effects of liquid molasses on animal production reported herein may be associated with amount of sugars close to exceed the threshold levels recommended for lactating dairy cows, particularly at the greatest level of supplementation, 12% liquid molasses. However, Brito et al. (2015) observed no differences in yields of milk and milk fat and protein in organic dairy cows fed corn meal or liquid molasses at 12% of the diet dry matter or 7.5% of added sugars. In addition, previous research in which high amounts of dried molasses—up to 12% of diet dry matter or 4.6% added sugars—or liquid molasses—up to 9% of diet dry matter or 7.4% added sugars—were fed to conventional dairy cows, milk yield responded cubically or quadratically (Broderick and Radloff 2004).

Overall, these inconsistent production results reported in the literature when feeding high dietary levels of molasses to lactating dairy cows may be related to differences in the ingredient composition of the basal diet, stage of lactation, and forage to concentrate ratios. The concentration of enterolactone in milk responded cubically to increasing amounts of liquid molasses as shown in Figure 1. It is unlikely that liquid molasses or corn meal would contribute with a significant amount of SDG that could explain this curvilinear response as flaxseed meal is the richest source of SDG. Therefore, based on this cubic effect, no conclusive answer about the impact of replacing corn meal with liquid molasses or the interaction of these 2 energy sources modulating the ruminal production of enterolactone or the concentration of this antioxidant in milk can be provided.

Conclusion

Replacing corn meal with liquid molasses decreased dry matter and yields of milk and milk components in a linear fashion, while milk enterolactone responded cubically. Based on the current results, it is not recommended to feed more than 8% of liquid molasses to lactating dairy cows. Further research is needed to better understand the interactions of liquid molasses and corn meal as potential modulators of enterolactone production in the rumen and appearance in milk.

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Appendix

Table 1. Ingredient and nutrient composition of the experimental diets

Ingredient composition, % of diet dry matter				
Mixed mostly grass baleage	31.2	31.2	31.2	31.2
Mixed mostly legume baleage	20.8	20.8	20.8	20.8
Grass hay	8.0	8.0	8.0	8.0
Liquid molasses	0.0	4.0	8.0	12.0
Corn meal	12.0	8.0	4.0	0.0
Flaxseed meal	15.0	15.0	15.0	15.0
Roasted soybean	2.5	2.5	2.5	2.5
Soybean hulls	8.5	8.5	8.5	8.5
Minerals-vitamins premix	2	2	2	2
Nutrient composition, % of dry matter (unless otherwise noted)				
Dry matter, % fresh matter	52.80	50.46	50.75	50.10
Crude protein	19.6	19.0	18.8	18.6
Neutral detergent fiber	44.2	43.7	43.3	42.8
Acid detergent fiber	28.8	28.7	28.5	28.3
Starch	9.69	7.01	4.33	1.64
Sugars[1]	0	1.65	3.30	4.95
Ash	6.84	7.16	7.28	7.81
Crude fat	4.20	4.06	3.93	3.79
NE_L[2], Mcal/kg	1.45	1.44	1.44	1.44

[1] Sugars from liquid molasses only.
[2] NE_L = Net energy for lactation.

Table 2. Least square means for dry matter intake, milk yield, and concentration and yields of milk components in organic dairy cows fed incremental amounts of liquid molasses

	Liquid molasses (% diet dry matter)					Contrasts (*P*-values)		
Item	0%	4%	8%	12%	SEM	Linear	Quadratic	Cubic
Dry matter intake, kg/d	19.1	18.6	18.4	17.6	0.68	0.05	0.96	0.93
Milk yield, kg/d	18.9	18.0	17.8	16.8	1.44	<0.01	0.94	0.41
Milk fat, %	5.28	5.37	5.28	5.25	0.16	0.51	0.42	0.63
Milk fat, kg/d	1.01	0.93	0.96	0.84	0.05	<0.01	0.32	0.54
Milk true protein, %	4.03	4.04	4.02	3.99	0.13	0.31	0.42	0.85
Milk true protein, kg/d	0.76	0.70	0.72	0.63	0.04	<0.01	0.70	0.52
Milk lactose, %	4.66	4.62	4.64	4.60	0.05	0.06	0.47	0.67
Milk lactose, kg/d	0.90	0.81	0.84	0.74	0.07	<0.01	0.80	0.32
Milk urea-N, mg/dL	16.8	16.4	15.8	16.0	0.54	0.04	0.25	0.52
Milk SCC[1], ×1,000 cells/mL	174	186	166	153	37.1	0.20	0.40	0.53

[1] SCC = somatic cells count

Figure 1. Concentration of enterolactone in milk of organic dairy cows fed incremental amounts of liquid molasses. Milk enterolactone averaged 246, 324, 251, and 324 n*M* in cows fed 0, 4, 8, or 12 % of liquid molasses, respectively; SEM = 62.1 n*M*; Contrasts *P*-values: Linear = 0.57, Quadratic = 0.58, and Cubic = 0.05.