Effects of a molasses-coated cottonseed product on diet digestibility, performance, and milk fatty acid profile of lactating dairy cattle¹

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ABSTRACT

An experiment was conducted to evaluate the effects of a molasses-coated cottonseed product on nutrient digestibility and milk fatty acid (FA) composition of lactating dairy cattle. The effect of a direct-fed microbial (DFM) product was also examined. Twelve Holstein cows (693 ± 85 kg of body weight, 127 ± 39 d in milk, 2.08 ± 0.29 lactations; mean $\pm$ SD) were randomly assigned to sequence in a replicated 4×4 Latin square design balanced for carryover effects. Cows were fed 1 of 4 treatments during each of the four 14-d periods: a control diet including 11.4% (dry matter basis) reginned cottonseed (CON), a diet with 14.4% molasses-coated cottonseed to match the cottonseed inclusion rate of the control diet (TC), the control diet with the addition of a liquid form of the cotton coating used to produce molasses-coated cottonseed (LC), and the LC diet with the addition of a DFM (LC+DFM). Diets were formulated for equal concentrations of neutral detergent fiber, crude protein, ether extract, and macrominerals. Treatments had no effect on dry matter intake, apparent total-tract nutrient digestibility, or milk production. The molasses coat, in either form, tended to decrease concentrations of odd-chain FA (2.25 and 2.31 vs. 2.35 g/100 g of FA for TC, LC, and CON, respectively) and unsaturated FA (31.4 and 31.1 vs. 32.1 g/100 g of FA) in milk. This could be indicative of a mild shift in ruminal fermentation away from propionate-producing bacteria toward fiber-digesting bacteria responsible for biohydrogenation of FA. The form of the molasses coating had few effects, but LC significantly decreased concentrations of total *trans*-C18:1 (2.04 vs. 2.30 ± 0.13 g/100 g of FA) and polyunsaturated FA (4.81 vs. 5.01 ± 0.17) compared with TC, implying that the liquid form slightly enhanced ruminal FA biohydrogenation. Furthermore, adding the DFM to the LC diet tended to increase the proportion of long-chain FA (FA >C16) and

significantly increased the proportions of *trans*-C18:1 (2.22 vs. 2.04 ± 0.13 g/100 g of FA) and unsaturated FA (32.4 vs. 31.1 ± 0.7 g/100 g of FA), suggesting an inhibitory effect on ruminal biohydrogenation. Results suggest that coating cottonseed with a hardened molasses product does not significantly depress nutrient digestibility and may provide a convenient method of incorporating these ingredients into dairy rations.

Key words: molasses, cottonseed, dairy cattle, direct-fed microbial

INTRODUCTION

Cottonseed is a highly valued commodity in dairy nutrition; however, its physical characteristics make it challenging to store and transport. One approach to solving this problem has been to coat cottonseed with starch, making the product more flowable while providing additional nutrients for the cow. Unfortunately, some evidence suggests that coating cottonseed with starch decreases nutrient digestibility (Solaiman et al., 2002) and milk fat concentration (Firkins et al., 2002), possibly because the coat limits bacterial access to fiber thus slowing digestion, or because of the negative associative effects of the additional starch provided in the product.

An alternative approach is to use a hardened molasses-based coating, which can offer handling and storage characteristics similar to starch-coated cottonseed while possibly enhancing nutritive value (Miller et al., 2005; Firkins et al., 2008). Researchers have observed that molasses has more favorable effects on the ruminal environment, especially for fiber digestion, compared with starch (Broderick and Radloff, 2004; Broderick et al., 2008). Ribeiro et al. (2005) observed an increase in bacterial OM production in response to added sucrose (0 to 8%) in continuous culture. More surprisingly, when dietary sucrose increased from 2.8 to 5.7%, ruminal pH increased (Penner et al., 2009), which can be beneficial to fiber-digesting microorganisms. These findings suggest that sucrose from dietary molasses could provide readily available energy without promoting subacute ruminal acidosis. Molasses products are commonly fed on dairy farms, and they also have stor-

Received October 6, 2009.

Accepted March 31, 2010.

¹Contribution no. 10-095-J from the Kansas Agricultural Experiment Station.

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age and transport challenges. Therefore, a molasses-coated cottonseed product could potentially replace 2 inconvenient commodities with a single product that is easier to handle.

Direct-fed microbials (DFM) are thought to improve feedstuff utilization by stimulating the growth of resident ruminal microbial populations. Among the microbial feed additives, fungi are thought to produce high levels of cellulases and xylanases (Akin and Borneman, 1990), which could increase fiber digestion in ruminant animals. One particular fungal strain, *Aspergillus oryzae*, has produced mixed results when incorporated into lactating cow diets, but has improved digestibility of various fiber fractions in some studies (Martin and Nisbet, 1992). Coupling *A. oryzae* with supplement molasses could have an additive effect to stimulate fiber digestion.

A molasses-coated cottonseed (MCC; Quality Liquid Feeds, Dodgeville, WI) product was recently developed, but its effects on animal performance have not yet been evaluated. Despite potential benefits of molasses on ruminal fermentation, a hardened molasses coat could limit access to the encased cottonseed and thereby limit digestion of fiber. As such, our primary aim was to assess total-tract digestion of nutrients in diets containing uncoated cottonseed compared with MCC. In addition, a DFM product containing a fermentation extract of *A. oryzae* was evaluated for its effects on nutrient digestibility in this type of diet.

MATERIALS AND METHODS

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

Design and Treatments

Twelve Holstein cows (693 ± 85 kg of BW, 127 ± 39 DIM, 2.08 ± 0.29 lactations; mean $\pm$ SD) were randomly assigned to square and sequence within square in a replicated 4×4 Latin square design balanced for carryover effects. Animals were randomly assigned to 1 of 12 stalls in a tie-stall facility, milked twice daily (0500 and 1600 h), and fed twice daily (0630 and 1700 h) for ad libitum intake. Treatment periods were 14 d, with 10 d for diet adaptation and 4 d for sample and data collection. Recombinant bST (Posilac, Monsanto, St. Louis, MO) was administered on d 1 of each period.

Cows were offered 1 of 4 dietary treatments: a control diet including 11.4% (DM basis) reginned cottonseed (CON); a treatment cotton diet with 14.4% MCC to match the cottonseed inclusion rate of the control diet (TC); the control diet with addition of a liquid form

of the cotton coating used to produce MCC (LC); and the LC diet with the addition of a DFM (LC+DFM). Composition and nutrient densities for the experimental diets are shown in Table 1. Diets were formulated for equal concentrations of NDF, CP, ether extract (EE), and macrominerals. The DFM was an extract of *A. oryzae* (Amaferm, BioZyme Enterprises Inc., St. Joseph, MO) and was fed at a rate of 57 g/cow per d. Reginned cottonseed used in the CON, LC, and LC+DFM diets was from the same batch as used in the production of MCC. A common base mix of alfalfa hay, corn silage, and corn gluten feed was prepared daily and used in all diets. All other ingredients, with the exception of cottonseed, MCC, liquid molasses coating, and the DFM, were included in grain mixes that were prepared for each respective treatment (LC and LC+DFM shared a common grain mix). Premixes, treatment ingredients, and the DFM were mixed into a TMR once daily in a research-scale feed mixer (Data Ranger, American Calan, Northwood, NH).

Data and Sample Collection

During the final 4 d (d 11 to 14) of each period, milk yield was recorded and 2 milk samples were collected at each milking. One sample was used for component analysis and the other was stored at -20°C for analysis of fatty acid (FA) profile. Feed offered and refused was measured for each cow on d 11 to 14 of each period to determine DMI. Samples of each cow's orts (12.5%) and each feed ingredient were collected and composited by period. From d 11 to 14, fecal samples (500 g) were collected from each cow every 9 h and frozen; this sampling schedule represented every 3 h of a 24-h period to account for possible diurnal variation in fecal composition.

Sample Analyses

One set of milk samples was analyzed by Heart of America DHIA (Manhattan, KS) to determine concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), urea nitrogen (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments). Fat-corrected milk (3.5%) was calculated according to the equation ($0.434 \times \text{milk yield} + 16.216 \times \text{milk fat yield}$). The second set of milk samples was used for FA analysis essentially as described (Sukhija and Palmquist, 1988). Milk samples were thawed, shaken, and composited into 1 sample/cow per period. The composited samples (≈ 200 μL) were lyophilized, resuspended in 1 mL of benzene containing C19:0 as an internal standard, and methylated using BF_3 -methanol. The resulting FA

Table 1. Ingredient and nutrient composition of experimental diets¹

Item	Diet ²			
	CON	TC	LC	LC+DFM
Ingredient				
Alfalfa hay	25.1	25.0	25.2	25.2
Corn silage	25.1	25.0	25.2	25.2
Corn gluten feed	11.2	11.2	11.3	11.3
Reginned cottonseed	11.4	—	11.4	11.4
MCC ³	—	14.4	—	—
Liquid cotton coat	—	—	1.9	1.9
Corn grain	16.4	16.6	15.3	15.3
Expeller soybean meal	4.9	4.9	4.9	4.9
Soybean meal (48% CP)	3.4	0.9	2.4	2.4
Limestone	1.03	0.63	1.08	1.08
Sodium bicarbonate	0.62	0.60	0.74	0.74
Magnesium oxide	0.12	0.14	0.10	0.10
Dicalcium phosphate	0.14	—	—	—
Sodium chloride	0.10	0.10	—	—
Sulfur	0.03	—	0.03	0.03
Micronutrient premix ⁴	0.51	0.51	0.52	0.52
Direct-fed microbial	—	—	—	0.06
Nutrient				
DM	63.7	63.6	63.2	63.2
CP	18.0	18.1	18.3	18.3
RUP, ⁵ % of CP	36.2	34.3	34.7	34.7
NDF	34.2	34.9	34.1	34.2
ADF	21.3	21.5	21.2	21.2
Starch	20.3	19.8	21.3	21.3
Total sugars ⁶	4.7	5.1	6.0	6.0
Ether extract	4.5	4.5	4.5	4.5
Ash	10.6	10.2	10.1	10.1
Acid insoluble ash	0.69	0.72	0.68	0.68

¹Values other than DM and RUP are expressed as a percentage of diet DM.

²CON = control diet; TC = treatment cotton diet; LC = liquid coating diet; LC+DFM = liquid coating diet + direct-fed microbial.

³Molasses-coated cottonseed.

⁴Premix included 67.1% trace mineral premix, 13.4% 4-Plex (Zinpro Corp., Eden Prairie, MN), 9.4% selenium premix, 2.7% vitamin A premix, 0.7% vitamin D premix, and 6.7% vitamin E premix.

⁵Estimated according to NRC (1989).

⁶Reducing sugar analysis following invertase incubation.

methyl esters were extracted in hexane and injected onto a Supelco SP-2560 capillary GC column (100 m × 0.25 mm × 0.2 µm; Supelco, Bellefonte, PA) for FA profile analysis, with a run time of 67 min. It should be noted that the procedure used to quantify FA in this experiment prevented detection of C4:0, which typically makes up around 5% of FA in milk (Jensen, 2002). Proportions reported are as a percentage (g/100 g) of total FA detected in this analysis.

Feed and orts samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA) and then composited by period. Fecal samples were dried at 55°C in a forced-air oven for 72 h and ground through a 5-mm screen; then, fecal samples from a single cow period were composited on an equal mass basis and ground through a 1-mm screen. Feed, orts, and fecal samples

were analyzed for ash, CP, NDF, ADF, acid detergent insoluble ash (**ADIA**), EE, and starch. Crude protein was determined by oxidation and detection of elemental nitrogen (Leco Analyzer, Leco Corp., St. Joseph, MI). Ash concentrations were determined after 4 h of oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined (Van Soest et al., 1991) with an Ankom apparatus (Ankom Technology, Macedon, NY) including the use of heat-stable α-amylase after extracting lipids with acetone; ADF and ADIA were also determined according to Van Soest et al. (1991) with an Ankom apparatus. Starch was analyzed as described by Herrera-Saldana and Huber (1989), and free glucose was quantified by the glucose oxidase method (Raabo and Terkildsen, 1960). Total sugars were quantified for TMR samples by incubation with invertase followed by measurement of reducing sugars (Lane and Eynon, 1923). Nutrient concentrations other than DM

were expressed as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h. Apparent total-tract nutrient digestibilities were determined using ADIA as an internal marker (Cochran et al., 1986).

Statistical Analysis

Data were analyzed using mixed models (JMP, version 6.0, SAS Institute, Cary, NC) including the fixed effect of treatment and the random effects of period and cow. Somatic cell data were log-transformed before analysis to ensure a normal residual distribution. Values were deemed outliers and omitted from analysis when residuals were greater than 3 standard deviations from the mean. Two cow periods were removed from analysis of digestibility data because of outlier analysis; erroneous data from these cow periods was likely because of inaccurate ADIA values. Three preplanned contrasts were tested for each variable of interest. Contrast 1 evaluated the effect of dietary molasses (TC and LC vs. CON), contrast 2 evaluated the effect of molasses form (TC vs. LC), and contrast 3 evaluated the effect of a DFM (LC+DFM vs. LC). Significance was declared at $P < 0.05$, and tendencies were declared at $P < 0.10$.

In Situ Digestibility Determination

To further evaluate the potential effects of MCC on digestibility, MCC and reginned cottonseed were used in an in situ digestion experiment. Digestion of DM and NDF were determined after 24 h of ruminal incubation; a mean residence time of 24 h corresponds to a passage rate of approximately 2.8%/h, which is similar to the NDF passage rates reported for lactating Holstein cows with DMI similar to those included in the current study (Taylor and Allen, 2005). Three nonlactating Holstein cows fitted with ruminal cannulas were kept in a single pen and had free access to feed and water, which were consumed ad libitum. Cows were fed a high-fiber, dry-cow ration and had been receiving it for at least 3 wk before the start of the trial.

Approximately 250 g of MCC and reginned cottonseed were placed in separate sealable plastic bags. Seeds were spread into a single layer and cracked by striking the bag with a hammer. Seeds were cracked rather than ground so that the seed contents would be available to ruminal degradation but the molasses coat would remain essentially intact. Cracked cottonseeds (3 g) were placed in polyester in situ bags (5 × 10 cm) with a pore size of 50 µm. Bags were sealed initially within the top 1 cm with an Ankom heat sealer, and a second seal was added directly below in the same manner. Each of the 3 animals had 10 bags of MCC and 10 bags of the

Table 2. Analysis of treatment ingredients¹

Item	Reginned cottonseed	Molasses-coated cottonseed
DM	89.0	87.0
CP	25.9	26.5
NDF	42.5	39.9
ADF	32.5	28.5
Starch	1.8	1.5
Ether extract	19.4	15.6
Ash	16.3	18.0
Acid detergent insoluble ash	0.14	0.41
In situ DM disappearance ²	57.4	62.2
In situ NDF disappearance ²	11.1	12.7

¹Nutrients other than DM are expressed as a percentage of DM. Standard errors of the means: DM, 1.9; NDF, 1.7.

²Determined after 24-h ruminal incubation. Values are expressed as a percentage and are not significantly different ($P > 0.10$).

reginned cottonseed added to a weighted mesh bag and placed in the rumen. After 24 h, all bags were removed from the animals and washed. Samples were dried in a 55°C forced-air oven for 72 h to determine DM. Dried samples and 10 samples of each product not subjected to ruminal degradation were analyzed for NDF content as described above. Disappearance of NDF and DM were calculated and statistically analyzed with a model including the fixed effect of feedstuff and the random effect of cow.

RESULTS AND DISCUSSION

Feedstuff Characterization and Digestibility

Results of proximate analysis and in situ digestibility determinations for reginned cottonseed and MCC are shown in Table 2. The molasses coating used to create MCC contained minerals to aid in the hardening process; therefore, MCC had increased ash content compared with the control cotton. The additional molasses also included nonprotein nitrogen, so the CP content of MCC was slightly higher than that of the control. However, because of dilution, coating the cottonseed led to decreased concentrations of NDF, ADF, and EE.

In situ digestibility analysis was carried out in unground feed samples to assess whether the molasses coat negatively affected fiber digestibility by limiting access to the cottonseed. However, determining the in situ digestibility of NDF in unground cottonseed products proved challenging. Samples of the products that were not subjected to ruminal degradation were initially analyzed for NDF concentration. Although the NDF content of reginned cottonseed matched with values determined after grinding (42.7 vs. 42.5%), the MCC values were clearly inaccurate (50.7 vs. 39.9%). The higher value obtained from unground MCC is inaccurate because the molasses coating should dilute NDF com-

Table 3. Effects of treatments on apparent total-tract nutrient digestibility (%)

Item	Treatment ¹				SEM	Contrast <i>P</i> -value ²		
	CON	TC	LC	LC+DFM		Mol	Form	DFM
OM	82.4	82.1	83.0	82.1	0.7	0.80	0.37	0.35
NDF	73.7	74.0	74.6	72.8	1.0	0.58	0.68	0.14
ADF	73.1	72.4	72.6	71.3	0.9	0.49	0.90	0.24
Starch	93.9	94.5	94.8	95.8	1.4	0.34	0.78	0.29
CP	84.4	83.2	84.3	83.8	0.7	0.34	0.28	0.62
EE ³	91.0	90.2	91.1	90.7	0.6	0.31	0.20	0.74

¹CON = control diet; TC = treatment cotton diet; LC = liquid coating diet; LC+DFM = liquid coating diet + direct-fed microbial.

²Contrasts: Mol = effect of molasses (TC and LC vs. CON); Form = effect of form of molasses (TC vs. LC); DFM = effect of the direct-fed microbial (LC+DFM vs. LC).

³Ether extract.

pared with reginned cottonseed, so the expected NDF content for MCC is close to the 39.9% of DM obtained with the ground sample. This suggests that the NDF procedure alone is not adequate to solubilize the molasses coat and expose the entire seed to neutral detergent extraction. However, samples that were subjected to 24 h of in situ degradation had NDF disappearance similar to that of the reginned cottonseed (Table 2). This is not a perfect method for determining ruminal availability or digestibility of fiber from cottonseed, but the results do not suggest that NDF digestibility of cottonseed is decreased by the molasses-based coat.

Apparent total-tract digestibilities of OM, NDF, ADF, starch, CP, and EE are shown in Table 3. No significant effects of treatment were detected for any of the nutrients measured. Although adding dietary molasses can improve nutrient digestibility (Huhtanen, 1988; Broderick and Radloff, 2004), particularly of fiber (Galloway et al., 1991; Heldt et al., 1999), differences were not observed in this study. Nevertheless, these re-

sults provide evidence that the molasses-based coating does not dramatically decrease nutrient digestibility in a TMR.

Animal Performance

Diets evaluated in this study had no significant effects on DMI, milk production, milk component concentration or yield, crude feed efficiency, or SCC in milk (Table 4). The lack of differences across treatments is similar to observations when a starch-coated cottonseed was fed (Firkins et al., 2002), except that the molasses coating did not decrease milk fat concentration. However, it should be noted that 14-d periods were used in this experiment, and the relative production responses may have been different had diets been fed for an extended period.

Adding the DFM to the LC diet tended to decrease MUN concentration, but the reason for this response was not clear because protein intake, digestibility, and

Table 4. Effects of treatments on performance of lactating cows

Item	Treatment ¹				SEM	Contrast <i>P</i> -value ²		
	CON	TC	LC	LC+DFM		Mol	Form	DFM
DMI, kg/d	29.2	28.8	29.1	29.2	0.8	0.55	0.50	0.78
Milk, kg/d	39.0	39.8	38.8	38.7	1.5	0.58	0.20	0.88
3.5% FCM, kg/d	39.9	40.7	39.9	40.2	1.6	0.67	0.26	0.61
3.5% FCM/DMI, kg/kg	1.37	1.42	1.37	1.38	0.05	0.48	0.16	0.73
Milk fat, %	3.64	3.66	3.63	3.72	0.09	0.97	0.68	0.31
Milk protein, %	3.15	3.12	3.12	3.11	0.07	0.23	0.99	0.77
Milk lactose, %	4.76	4.75	4.76	4.78	0.06	0.81	0.64	0.36
Milk fat, kg/d	1.42	1.46	1.41	1.45	0.06	0.70	0.32	0.44
Milk protein, kg/d	1.22	1.23	1.21	1.20	0.03	0.84	0.33	0.84
Milk lactose, kg/d	1.87	1.89	1.86	1.86	0.09	0.86	0.41	0.99
MUN, mg/dL	15.6	15.7	16.0	15.4	0.6	0.47	0.31	0.09
SCC, log	4.64	4.84	4.70	4.45	0.40	0.58	0.63	0.34

¹CON = control diet; TC = treatment cotton diet; LC = liquid coating diet; LC+DFM = liquid coating diet + direct-fed microbial.

²Contrasts: Mol = effect of molasses (TC and LC vs. CON); Form = effect of form of molasses (TC vs. LC); DFM = effect of the direct-fed microbial (LC+DFM vs. LC). Significant contrast *P*-values and tendencies are bolded.

Table 5. Effects of treatments on milk fatty acid profile

Fatty acid, ¹ g/100 g	Treatment ²					Contrast <i>P</i> -value ³		
	CON	TC	LC	LC+DFM	SEM	Mol	Form	DFM
C6:0	2.53	2.63	2.55	2.54	0.08	0.27	0.13	0.92
C8:0	1.47	1.50	1.48	1.44	0.06	0.41	0.61	0.25
C10:0	3.12	3.16	3.19	3.05	0.13	0.49	0.78	0.13
C11:0	0.38	0.35	0.36	0.35	0.02	0.11	0.22	0.35
C12:0	3.44	3.44	3.50	3.39	0.15	0.70	0.52	0.27
C14:0	10.9	11.0	11.0	10.8	0.2	0.38	0.96	0.23
C14:1	0.75	0.72	0.75	0.76	0.06	0.51	0.30	0.70
C15:0	1.08	1.02	1.05	1.04	0.03	0.08	0.46	0.82
C16:0	28.8	29.1	29.3	28.7	0.6	0.13	0.39	0.02
C16:1	1.51	1.46	1.47	1.55	0.07	0.08	0.55	0.03
C17:0	0.89	0.88	0.90	0.90	0.01	0.49	0.14	0.71
C18:0	15.0	15.2	15.3	15.1	0.5	0.42	0.83	0.66
C18:1n9t	0.37	0.41	0.36	0.38	0.01	0.02	0.001	0.08
C18:1n10t	0.60	0.65	0.56	0.62	0.05	0.73	0.004	0.05
C18:1n11t	1.22	1.23	1.12	1.22	0.08	0.38	0.06	0.09
C18:1n9c	22.0	21.4	21.5	22.3	0.6	0.09	0.88	0.03
C18:1n11c	0.56	0.51	0.55	0.56	0.03	0.10	0.17	0.48
C18:2n6t	0.028	0.027	0.034	0.027	0.003	0.44	0.15	0.15
C18:2n6c	3.38	3.43	3.24	3.30	0.13	0.36	0.001	0.22
C20:0	0.18	0.18	0.18	0.18	0.01	0.70	0.44	0.92
C18:3n6	0.064	0.064	0.061	0.063	0.004	0.54	0.24	0.50
C20:1	0.033	0.032	0.029	0.034	0.002	0.26	0.27	0.08
C18:3n3	0.38	0.39	0.37	0.38	0.02	0.51	0.03	0.33
CLA 9c,11t	0.44	0.44	0.40	0.46	0.03	0.33	0.13	0.03
CLA 10t,12c	0.000	0.001	0.000	0.003	0.002	0.73	0.55	0.20
CLA 9c,11c	0.007	0.010	0.006	0.003	0.003	0.90	0.28	0.44
CLA 9t,11t	0.056	0.056	0.054	0.059	0.003	0.78	0.49	0.13
C20:2	0.033	0.031	0.030	0.032	0.002	0.03	0.43	0.09
C22:0	0.059	0.058	0.057	0.056	0.003	0.54	0.76	0.67
C20:3n6	0.23	0.20	0.22	0.21	0.02	0.02	0.07	0.69
C20:4n6	0.23	0.21	0.22	0.21	0.01	0.02	0.37	0.40
C23:0	0.018	0.024	0.024	0.019	0.004	0.07	0.91	0.19
C20:5n3	0.044	0.040	0.045	0.047	0.004	0.76	0.36	0.65
C22:5n3	0.12	0.10	0.13	0.13	0.02	0.73	0.17	0.82
C22:6n3	0.007	0.008	0.009	0.010	0.006	0.38	0.65	0.59

¹c = *cis*; t = *trans*; CLA = conjugated linoleic acid.

²CON = control diet; TC = treatment cotton diet; LC = liquid coating diet; LC+DFM = liquid coating diet + direct-fed microbial.

³Contrasts: Mol = effect of molasses (TC and LC vs. CON); Form = effect of form of molasses (TC vs. LC); DFM = effect of the direct-fed microbial (LC+DFM vs. LC). Significant contrast *P*-values and tendencies are bolded.

milk protein yield were unchanged by this treatment. Reports have noted that addition of fungal DFM leads to lower ruminal ammonia concentration (Yoon and Stern, 1995), which is possibly related to increased ammonia utilization that occurs because of greater ruminal microorganism populations. Because a substantial portion of MUN is derived from ruminal ammonia being converted to urea in the liver, it is possible that DFM effects on MUN are explained by a ruminal mechanism.

Milk Fatty Acids

It is well known that rumen microbial populations are influenced by diet composition and some of these species play a role in biohydrogenation of dietary lipids (Jenkins et al., 2008). Milk FA profiles provide a nonin-

vasive means of probing ruminal metabolism by quantifying shifts in biohydrogenation intermediates as well as lipogenic substrates (i.e., acetate vs. propionate). Milk FA profiles are shown in Table 5.

Fatty acid profile data were compiled into classes of FA to ease interpretation of results (Table 6). Addition of molasses (in the form of a coating or liquid) decreased the proportion of odd-chain FA (C11, C15, C17) and tended to decrease the proportion of unsaturated FA in milk fat. Odd-chain FA are typically produced when propionate, rather than acetate, is used as a precursor for FA synthesis in the mammary gland (Vlaeminck et al., 2006) and can be used to assess relative differences in plasma propionate concentration. The effect of molasses may reflect a minor shift in ruminal fermentation away from propionate production and, presumably, toward greater production of acetate and butyrate. The ten-

Table 6. Effects of treatments on classes of milk fatty acids (FA)

Item	Treatment ¹				SEM	Contrast <i>P</i> -value ²		
	CON	TC	LC	LC+DFM		Mol	Form	DFM
Short- and medium-chain FA, ³ %	23.6	23.9	23.9	23.4	0.6	0.56	0.90	0.25
C16 FA, %	30.3	30.5	30.7	30.3	0.6	0.16	0.31	0.09
Long-chain FA, ⁴ %	46.0	45.6	45.4	46.4	0.9	0.31	0.61	0.08
Odd-chain FA, %	2.35	2.25	2.31	2.29	0.05	0.05	0.17	0.72
Total <i>trans</i> -C18:1, ⁵ %	2.19	2.30	2.04	2.22	0.13	0.79	0.005	0.05
Polyunsaturated FA, %	5.01	5.01	4.81	4.94	0.17	0.14	0.02	0.12
Total unsaturated FA, %	32.1	31.4	31.1	32.4	0.7	0.06	0.51	0.01
Δ^9 -Desaturase index, ⁶ %	6.4	6.1	6.3	6.6	0.4	0.22	0.32	0.26

¹CON = control diet; TC = treatment cotton diet; LC = liquid coating diet; LC+DFM = liquid coating diet + direct-fed microbial.

²Contrasts: Mol = effect of molasses (TC and LC vs. CON); Form = effect of form of molasses (TC vs. LC); DFM = effect of the direct-fed microbial (LC+DFM vs. LC). Significant contrast *P*-values and tendencies are bolded.

³Fatty acids <C16.

⁴Fatty acids >C16.

⁵Includes *trans*-9, *trans*-10, and *trans*-11 C18:1.

⁶Calculated as C14:1/(C14:0 + C14:1).

dency for an effect on unsaturated FA seems to support this interpretation, as acetate-producing bacteria are thought to be important for ruminal biohydrogenation of unsaturated FA (Harfoot and Hazlewood, 1988; Nam and Garnsworthy, 2007a). The Δ^9 -desaturase index was not altered by diet (Table 6), suggesting that effects on milk fat saturation were due to ruminal effects.

Interestingly, LC significantly decreased the proportions of both polyunsaturated and *trans*-C18:1 FA in milk compared with TC. Although we are unaware of any studies examining the effects of dry versus liquid molasses on biohydrogenation, the decreased proportions of these classes of unsaturated FA suggest that the liquid molasses product enhanced ruminal biohydrogenation to a greater extent than did the dried form in the TC diet. This effect on milk FA profile could also be related to the greater sugar content in the LC diet (Table 1) creating a more favorable pH (Penner et al., 2009) or providing more favorable substrates (Cotta, 1992) for biohydrogenating bacteria.

Finally, adding the fungal DFM to the LC diet significantly increased the proportions of *trans*-C18:1 and total unsaturated FA in milk and tended to increase the proportion of long-chain FA in milk. As for molasses treatments, the DFM did not alter Δ^9 -desaturase index, suggesting that effects on FA profile were caused by differences in ruminal metabolism. The increase in unsaturated FA may indicate that the DFM had an inhibitory effect on ruminal biohydrogenation, although to our knowledge, such an effect has not been previously reported. Ruminal fungi have the ability to biohydrogenate, but biohydrogenation in fungi is much slower than in ruminal bacteria (Nam and Garnsworthy, 2007a,b). Thus, it is possible that the additional ruminal fungi inhibited bacterial access to substrate,

leading to less biohydrogenation by bacteria. Although one would expect these additional microbes to contribute to biohydrogenation, a slower rate of biohydrogenation may have allowed for greater flow of unsaturated FA out of the rumen.

Although numerous effects of diet on FA profile were observed, none of the treatment effects were as dramatic as those observed in situations such as diet-induced milk fat depression (Kadegowda et al., 2008). This is not surprising, given the similarities in milk fat concentration and yield across treatments. Nonetheless, the small differences noted have biological significance in fermentation stoichiometry. Overall, milk FA profiles suggest that dietary molasses leads to a mild shift in ruminal fermentation away from propionate-producing bacteria toward fiber-digesting bacteria involved in biohydrogenation. The form of the molasses coating had few effects on variables measured in this study, but milk FA profiles suggest that the liquid form slightly enhanced ruminal FA biohydrogenation. Furthermore, addition of a DFM significantly increased the proportion of unsaturated FA in milk, which may indicate an inhibitory effect on ruminal biohydrogenation.

CONCLUSIONS

Coating reginned cottonseed with a molasses-based product did not alter DMI, in situ NDF digestibility, apparent total-tract nutrient digestibility, or productivity in lactating dairy cows. The added molasses tended to alter milk FA profiles in a manner suggesting that molasses promoted more extensive ruminal biohydrogenation. Incorporating the DFM increased amounts of unsaturated FA in milk, suggesting that it may have inhibited ruminal biohydrogenation. These data failed

to show any evidence that coating cottonseed with hardened molasses impairs nutrient digestibility or ruminal biohydrogenation, and this approach to improving the handling characteristics of cottonseed appears to be worthy of continued investigation.

ACKNOWLEDGMENTS

The authors express their appreciation to Jeff Koch and Mike Scheffel (Kansas State University, Manhattan) for assisting with this trial and to Quality Liquid Feeds (Dodgeville, WI) for partially funding this research.

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