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Fat and starch as additive risk factors for milk fat depression in dairy diets containing corn dried distillers grains with solubles

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ABSTRACT

Two experiments were conducted to evaluate the additive effects of starch and fat as risk factors associated with milk fat depression in dairy diets containing corn dried distillers grains with solubles. In experiment 1, 4 multiparous ruminally cannulated Holstein cows, averaging 114 ± 14 d in milk and 662 ± 52 kg of body weight, were randomly assigned to 4 treatments in a 4 × 4 Latin square to determine the effect of these risk factors on rumen fermentation and milk fatty acid profile. In each 21-d period, cows were assigned to 1 of 4 dietary treatments: a control diet (CON; ether extract 5.2%, starch 19%); CON with added oil (OL; ether extract 6.4%, starch 18%); CON with added starch (STR; ether extract 5.5%, starch 22%); and CON with added oil and starch (COMBO; ether extract 6.5%, starch 23%). After completion of experiment 1, milk production response was evaluated in a second experiment with a similar approach to diet formulation. Twenty Holstein cows, 12 primiparous and 8 multiparous, averaging 117 ± 17 d in milk and 641 ± 82 kg, were used in replicated 4 × 4 Latin squares with 21-d periods. Results from experiment 1 showed that ruminal pH was not affected by treatment averaging 5.87 ± 0.08. Molar proportion of propionate in rumen fluid was greatest on the COMBO diet, followed by OL and STR, and lowest for CON. The concentration of *trans*-10,*cis*-12 conjugated linoleic acid in milk fat increased with the COMBO diet. Adding oil, starch, or a combination of both resulted in lower concentration and yield of fatty acids <16 carbons. Compared with the control, OL and STR resulted in 13% lower concentration, whereas the COMBO diet resulted in a 27% reduction; similarly yield was reduced by 24% with the OL and STR treatments and 54% with the COMBO diet. In experiment 2, milk yield, milk protein percentage, and milk protein yield were similar across treatments, averaging 26.6 ± 1.01 kg/d, 3.2 ± 0.05%, and 0.84 ± 0.03 kg/d, respectively. Fat-corrected milk was greatest for CON, 26.5 ± 1.12 kg/d; no differences were detected among the remaining treatments, which averaged 23.5 ± 1.12 kg/d. Milk fat percentage was greatest when cows consumed CON, 3.3 ± 0.15%; OL and STR averaged 3.0 ± 0.15% and COMBO resulted in the lowest milk fat percentage, 2.73 ± 0.15%. Milk fat yield was 0.25 ± 0.05 kg/d greater for the CON diet compared with the other 3 treatments, which were similar. These results suggest that fat and starch are additive risk factors that will likely induce milk fat depression in diets containing high inclusion of dried distillers grains with solubles.
Key words: corn milling co-product, milk fat, milk fatty acid

INTRODUCTION

The production of ethanol from corn has rapidly grown, and, as a result, more animal feed co-products are available for the dairy industry (Renewable Fuels Association, 2013). Dried distillers grains with solubles (**DDGS**) are one example of these co-products and are usually a cost-effective source of protein (Liu, 2011; Paz et al. 2013) and energy for ruminants (Ham et al., 1994). Research has shown that dairy diets may contain 20% DDGS (DM basis) while maintaining or even increasing milk yield (Anderson et al., 2006; Kelzer et al., 2009; Schingoethe et al., 2009). Conversely, some authors have reported milk fat depression (**MFD**) when feeding DDGS (Abdelqader et al. 2009). Therefore, in commercial settings, the inclusion of this ingredient may be minimized to avoid MFD (Janicek et al., 2008; Hollmann et al., 2011).

Milk fat depression is a disorder characterized by normal milk production with low milk fat concentration (Bauman et al., 2008). This condition may develop due to the production and accumulation of bioactive isomers of CLA in the rumen during microbial biohydrogenation. Among others, the *trans*-10,*cis*-12 CLA isomer has been reported to be a potent suppressor of

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mammary uptake and de novo synthesis of FA (Chouinard et al., 1999; Baumgard et al., 2000). Furthermore, flow of these FA out of the rumen is increased when cows consume increasing amounts of long-chain PUFA (Bauman and Griinari, 2003). Additionally, Kalscheur et al. (1997a) reported MDF and increased production of *trans*-10 C18:1, a putative source of *trans*-10,*cis*-12 CLA, with diets high in starch from ground corn which lead to low ruminal pH. Because they contain a high concentration of PUFA, DDGS are often believed to be associated with MFD. However, it is likely that the extent to which DDGS cause MFD is related to other ingredients included as well as the type of fat (Kalscheur et al., 1997b) in the ration. Therefore, we hypothesized that feeding DDGS in a TMR with high starch content may predispose cows to undergo MFD due to increased ruminal fermentation and acidosis. Furthermore, the addition of corn oil would exacerbate this response by altering biohydrogenation of PUFA. Thus, the present study was designed to investigate the effects of increasing starch and PUFA to a control diet containing DDGS on rumen fermentation, milk production, and composition.

MATERIALS AND METHODS

Animal Care, Housing, and Sampling

Our study involved 2 experiments in which the experimental cows were cared for according to the guidelines stipulated by the University of Nebraska-Lincoln Animal Care and Use Committee. The following conditions were identical in both experiments. Cows were housed in individual stalls and milked at 0730 and 1930 h. Cows were individually fed at 0900 h for approximately 110% ad libitum consumption. Orts were collected, weighed, and recorded individually. Day 1 to 14 of each period were considered as an adaptation period; data collected during the last 7 d were considered for statistical analyses. Body weight and BCS (1 to 5 scale) were measured on d 20 and 21 of each period. Body condition score was measured by a single, trained individual, and the scoring method used was similar to that of Wildman et al. (1982) but reported to the quarter point.

Experiment 1: Animal, Experimental Design, and Treatments

A ruminal fermentation study was conducted with 4 multiparous ruminally cannulated Holstein cows averaging (±SD) 114 ± 14 DIM and 662 ± 52 kg of BW in a 4 × 4 Latin square with 21-d periods. Cows were randomly assigned to 1 of 4 experimental treatments (on a DM basis): a control diet (**CON**), 2 other treatments similar to CON but containing 0.97% corn oil (**OL**) or 8.5% additional ground corn (**STR**) as risk factors for MFD, and a fourth treatment containing 0.97% corn oil and 7.6% additional ground corn (**COMBO**) as a combination of risk factors. All diets contained DDGS at 20% (DM basis; Table 1). The forage-to-concentrate ratio of all diets was 53:47 with 23% forage NDF (as a % of dietary DM). When formulating the OL, STR, and COMBO treatments, soy hulls were removed from the CON formulation to allow inclusion of corn oil and ground corn.

Sampling and Data Collection

Feed Sampling. Research farm employees collected data on a daily basis, whereas feed sampling was performed by the researchers to coincide with rumen sampling and optimize resources. Samples of each TMR and forages were collected on d 20 and 21 of each period and subsequently pooled by period. The Penn State Forage Particle Separator (Nasco, Fort Atkinson, WI) was used to measure particle size distribution of the different TMR as described by Kononoff et al. (2003). Feed samples were dried at 55°C in a forced-air oven to determine DM. After determination of DM, samples were ground (1-mm screen; Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and stored at room temperature. Concentration of FA and profile were determined by GC-flame-ionization detection after direct methylation (Sukhija and Palmquist, 1988) on composite TMR samples using C13:0 or C17:1 (NuChek Prep Inc., Elysian, MN) as internal standards, as described by Rico et al. (2014).

Milk Data Collection. Individual milk production was measured and recorded daily by an automatized computer program for data collection; measurements from the last 7 d of each period were used to evaluate milk production; additionally, milk samples were collected during the morning and evening milking of d 19, 20, and 21 by farm employees and preserved using a pellet of 2-bromo-2-nitropropane-1,3-diol. Milk samples were analyzed for fat, true protein, lactose, and SNF (AOAC International, 2000) by Heart of America DHIA (Manhattan, KS) using a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN). Milk urea nitrogen was determined by the same laboratory using a modified Berthelot reaction concentration using a ChemSpec 150 Analyzer (Bentley Instruments). Yields of milk components were estimated according to milk weight and time of collection. During the last 7 d of each period, daily DMI and milk yield were averaged. An additional milk sample was taken at the times previously described for determination of FA profile. Individual samples were frozen immediately after milking,

Table 1. Ingredient and analyzed nutrient composition of experimental diets

	Dietary treatment[1]							
	Experiment 1				Experiment 2			
Item	CON	OL	STR	COMBO	CON	OL	STR	COMBO
Diet ingredient, % of DM								
Corn silage	33.4	33.4	33.4	33.4	31.0	31.0	31.0	31.0
Dried distillers grains with solubles	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9
Alfalfa haylage	9.7	9.7	9.7	9.7	7.4	7.4	7.4	7.4
Soybean hulls	8.5	7.6	—	—	8.5	7.6	—	—
Ground corn	7.8	7.8	7.8	7.8	12.6	12.6	12.6	12.6
Grass hay	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
Alfalfa hay	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Corn oil	—	1.0	—	1.0	—	1.0	—	1.0
Added ground corn	—	—	8.5	7.6	—	—	8.7	7.8
Cottonseed	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Soypass[2]	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Soybean meal	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Blood meal	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Limestone	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Sodium bicarbonate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Vitamin premix[4]	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Nutrient,[5] % of DM								
CP	17.4	17.9	17.7	17.2	16.2	16.9	16.0	16.4
NDF	41.0	39.0	39.0	37.0	40.0	39.0	33.0	31.0
Starch	19.2	18.1	22.1	23.3	19.4	19.7	25.6	23.7
Ether extract	5.2	6.4	5.5	6.5	5.5	6.2	5.4	6.2
NFC[6]	28.1	29.0	30.7	31.7	29.8	29.2	37.2	38.2
Ash	8.3	8.2	7.6	7.7	8.7	8.6	8.0	8.4
FA, g/100 g of DM								
C16:0	0.83	0.97	0.87	0.97	—	—	—	—
C18:0	0.14	0.16	0.14	0.15	—	—	—	—
C18:1 *cis*-9	1.15	1.50	1.26	1.48	—	—	—	—
C18:1 *cis*-11	0.04	0.05	0.04	0.05	—	—	—	—
C18:2 n-6	2.33	2.98	2.50	2.86	—	—	—	—
C20:0	0.02	0.03	0.03	0.03	—	—	—	—
C18:3 n-3	0.16	0.17	0.15	0.15	—	—	—	—
Other[7]	0.25	0.27	0.25	0.30	—	—	—	—
Total	4.93	6.14	5.24	6.00	—	—	—	—

[1]Experiment 1: CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% ground corn. Experiment 2: CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.7% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.8% ground corn.

[2]LignoTech, Overland Park, KS.

[3]Formulated to contained 1.0% Ca, 0.50% P, 0.36% Mg, and 1.3% K.

[4]Formulated to supply approximately 120,000 IU/d of vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d of vitamin E in the diet.

[5]Mean, n = 4.

[6]Calculated by 100 – (% NDF + % CP + % fat + % ash).

[7]Includes unknown FA and FA that were present at concentrations <0.02 g/100 g of DM (14:0, 14:1, 16:1, 17:0, 18:3, 20:1, 20:2, 22:0, 22:4, 24:0, 24:1).

and at the completion of the experiment 1 composite per cow in each period was obtained by mixing proportional aliquots according to milk weight and time of collection. Milk FA were analyzed as described by Rico and Harvatine (2013) with modifications. Briefly, milk fat was extracted with hexane:isopropanol, FAME was prepared by base-catalyzed transmethylation, and FAME were quantified by GC-FID (20:1 split ratio; initial oven temperature was 80°C, increased 2°C/min to 190°C and held for 15 min, and increased 5°C/min to 215°C and held for 3 min). Fatty acid peaks were identified in the gas chromatographic analysis using pure methyl ester standards (GLC 780, 68D, NuChek Prep Inc.; Bacterial Acid Methyl Ester Mix, 47080-U, Sigma-Aldrich Inc., St. Louis, MO; GLC-110, Matreya LLC., Pleasant Gap, PA). An equal-weight reference standard (GLC 461; NuChek Prep Inc.) was used to determine correction factors for individual FA.

Apparent Digestibility. Indigestible ADF was used as an internal marker to estimate apparent digestibility of nutrients (Huhtanen et al., 1994). Briefly, TMR and fecal samples were weighed (1.25 g) in quadruplicate into 5- × 10-cm Dacron bags with 50-μm pores (No. R510, Ankom Technology, Macedon, NY). Then, 2 bags of each sample were incubated in the rumen of 2 lactating cannulated cows fed a diet containing 60% forage and 40% concentrate for 12 d for indigestible ADF determination. Starting on d 18 to 21, fecal samples were collected before milking at 0600 and 1800 h by the rectal grab sample technique. Fecal samples from each collection time point (approximately 200 g) were immediately frozen (−20°C) for later analyses. Fecal samples were pooled to obtain a composite according to cow and period and then dried at 55°C in a forced-air oven and ground (1-mm screen; Wiley mill, Arthur H. Thomas Co.). Apparent digestibility of nutrients was estimated based on intake and concentration of the marker in feces to estimate fecal output.

Ground feed and fecal samples were analyzed for analytical DM (100°C oven for 24 h), nitrogen (Leco FP-528, Leco Corp., St. Joseph, MI), and percentage ash (AOAC International, 2000). Ether extract analysis (AOAC International, 2000) was done only on TMR samples by an external laboratory using diethyl ether as the solvent (Cumberland Valley Analytical Services, Hagerstown, MD); the same laboratory performed starch content analysis in feces and TMR samples according to Hall (2009). Determination of NDF was done according to Van Soest et al. (1991). Heat-stable α-amylase (500 μL per 0.5 g of sample; Sigma A3306, Sigma-Aldrich Inc.) and sodium sulfite (Fisher Chemical, Fair Lawn, NJ) were included in the NDF procedure on TMR samples (0.5 g/0.5 g of sample).

Rumen Sampling. On d 21 of each period, ruminal fluid samples were collected over 10 time points (0, 1, 2, 4, 6, 8, 11, 14, 18, and 23 h) postfeeding. Time points where chosen to accurately characterize diurnal rumen pH but to also coincide with daily milking, feeding times as well as the schedules of employees and students involved in the study. Rumen content samples of each cow were collected via the rumen cannula from the cranial, caudal, left lateral, and right lateral areas of the rumen and mixed to become uniform to obtain a representative sample per cow. This material was then strained through 4 layers of cheesecloth to obtain rumen fluid. Rumen fluid pH was measured immediately by using a hand-held pH electrode (model M90, Corning Inc., Corning, NY) and immediately stored in plastic, screw-capped 50-mL conical tubes at −20°C for later analyses. The samples were sent to an external laboratory for VFA analysis (Rumen Fermentation Profiling Laboratory, Morgantown, WV); briefly, analysis of VFA concentrations in effluents was performed according to the gas chromatographic separation procedure using trimethylacetic acid as the internal standard (Supelco, 1975). The gas chromatograph was a Varian model 3300 with an FID detector (Varian Inc., Palo Alto, CA). Working conditions were column temperature of 175°C, detector and injector temperature of 200°C, and N used as the carrier at a rate of 24 mL/min. The column was a 2-m × 2-mm glass column packed with 10% SP-1200/1% H_3HPO_4 on 80/100 chromosorb W AW (Supelco Inc., Bellefonte, PA).

Experiment 2: Animals, Experimental Design, and Treatments

After completion of experiment 1, a second experiment was conducted to evaluate the effect of similar diets on milk production using 12 primiparous and 8 multiparous Holstein cows (averaging 117 ± 17 DIM and 641 ± 82 kg) in balanced, replicated 4 × 4 Latin squares with 21-d periods. Animals were randomly assigned to 1 of 4 dietary treatments similar to those in the previous experiment (Table 1) with the following modifications: a portion of corn silage and alfalfa hay was removed to allow greater inclusion of ground corn. These changes resulted in diets with 49:51 forage-to-concentrate ratio and 21% NDF (as % of dietary DM). In this experiment we increased the inclusion level of supplemental ground corn to exacerbate the potential risk for MFD while targeting a common industry recommendation for starch between 25 and 30% of the dietary DM (Grant, 2005). Feed sampling, milk data collection, and animal measurements were performed as described in experiment 1.

Statistical Analyses

Experiment 1. Production data were analyzed as a 4 × 4 Latin square using the MIXED procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Treatment and period were considered as fixed effect in the model, whereas cow was considered as a random effect. The linear model for this experiment is written as

$$y_{ijk} = \mu + \beta_i + \rho_j + \alpha_k + \varepsilon_{ijk},$$

where y_{ijk} represents observation ijk; μ represents the overall mean; β_i represents the random effect of cow i; ρ_j represents the fixed effect of period j; and α_k represents the fixed effect of treatment k. The error term ε_{ijk} was assumed to be normally, independently, and identically distributed, with variance σ_e^2.

Data obtained from ruminal fluid were analyzed as repeated measures using the first-order antedependence

covariance structure in SAS (Version 9.2). The effects of period, treatment, hour, and treatment × hour interaction were considered as fixed and cow was considered as a random effect. The linear model for these data analysis is written as

$$y_{ijkm} = \mu + \beta_i + \rho_j + \gamma_k + \alpha_m + \gamma\alpha_{km} + \varepsilon_{ijkm},$$

where y_{ijkm} represents the observation ijkm; μ represents the overall mean; β_i represents the random effect of cow i; ρ_j represents the fixed effect of period j; γ_k represents the fixed effect of hour k; α_m represents the fixed effect of treatment m; and $\alpha\gamma_{km}$ represents the interaction between hour k and treatment m. The error term ε_{ijkm} was assumed to be normally, independently, and identically distributed, with variance σ_e^2. Due to unequally spaced rumen sampling, the weighted averages for rumen data were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972). Statistical significance for all treatments effects was declared at $P \leq 0.05$; trends are discussed at $P \leq 0.10$. All mean results are presented as least squares means ± the largest SEM unless stated otherwise.

Experiment 2. Production data were analyzed as balanced replicated 4 × 4 Latin squares using the MIXED procedure of SAS (Version 9.2). The fixed effects of the model included square, period within square, and treatment. Random effect included cow within square. The linear model for these data is written as

$$y_{ijkm} = \mu + \tau_m + \beta(\tau)_{im} + \rho(\tau)_{jm} + \alpha_k + \varepsilon_{ijkm},$$

where y_{ijkm} is the observation ijkm; μ represents the overall mean; τ_m represents the fixed effect square m; $\beta(\tau)_{im}$ represent the random effect of cow i within square m; $\rho(\tau)_{jm}$ represents the fixed effect of period j within square m; and α_k represents the fixed effect of treatment. The error term ε_{ijkm} was assumed to be normally, independently, and identically distributed, with variance σ_e^2. Statistical significance for all treatments effects was declared at $P \leq 0.05$; trends are discussed at $P \leq 0.10$. All mean results are presented as least squares means ± the largest SEM unless stated otherwise.

RESULTS

Forage and Diets Composition

Composition of forages and DDGS for each experiment is listed in Table 2. One single batch of DDGS was used for both experiments; average (±SD) values were 28.3 ± 0.4% CP, 11.9 ± 0.7% ether extract, and 31.3 ± 0.8% NDF. Particle size distribution of the TMR is presented on Table 3. Overall, retention of particles >19.0 mm averaged 7.2 ± 0.96%, particles retained in the 8.0- to 19.0-mm sieve averaged 26.7 ± 0.86% of the TMR. Particle size retention in the 1.18- to 8.0-mm sieve was 30.9 ± 0.72%. The proportion of particles <1.18 mm averaged 32.2 ± 1.11% across treatments.

DMI, Milk Production, and Composition

Production data for both experiments is presented in Table 4. The aim of the first experiment was to determine effects on ruminal fermentation, whereas experiment 2 aimed at determining effects on productive performance; therefore, production results are of greater focus in experiment 2. In the first experiment, daily DMI from cows receiving the CON, OL, and STR was similar, averaging 24.9 ± 1.24 kg, and was greater ($P < 0.01$) than that of cows consuming the COMBO diet, which averaged 22.0 ± 1.24 kg. In experiment 2, daily DMI and milk yield were not affected by treatment and averaged 22.2 ± 0.63 and 26.6 ± 1.01 kg, respectively. Milk performance was not affected by dietary treatment in experiment 1 except for milk fat. The COMBO diet resulted in the lowest ($P < 0.01$) concentration and yield of milk fat, 2.21 ± 0.18% and 0.69 ± 0.12 kg/d, respectively. Conversely, the CON diet resulted in the highest milk fat concentration, 3.19 ± 0.18%, and yield, 1.10 ± 0.12 kg/d. Cows receiving the OL and STR diets had intermediate values. Fatty acid profiles of milk are presented in Tables 5 and 6. The inclusion of oil, starch, or a combination of both reduced the overall concentration ($P = 0.02$) and yield ($P < 0.01$) of milk FA <16 carbons (de novo synthesized). Concentration of FA >16 carbons was lower ($P = 0.02$) for the control diet compared with the other 3 diets, 51.2, 57.5, 54.8, and 59.5 ± 1.91% for CON, OL, STR, and COMBO, respectively. Concentration of *trans*-10 18:1 increased ($P = 0.01$) with the addition of corn or oil, 0.62, 1.92, 1.58, and 3.44 ± 0.54 g/100 g for CON, OL, STR, and COMBO, respectively. In the same order, yield of this FA increased with addition of these risk factors, and mean daily yields were 6.31, 15.72, 10.86, 20.82 ± 3.29 g, respectively. Fatty acid profile of milk from cows consuming the COMBO diet showed that concentration of *trans*-10,*cis*-12 CLA was greater ($P = 0.05$) compared with the other diets in which concentration of this FA was negligible. In experiment 2, milk protein was not affected by treatment and overall means of concentration and yield were 3.17 ± 0.05% and 0.84 ± 0.03 kg, respectively; estimates of MUN for the CON, OL, and STR diets were similar at 11.1, 11.6, and 11.3, respectively, whereas the COMBO diet

Table 2. Nutrient composition of forages and corn milling by-product included in experimental diets

Chemical, % of DM (unless otherwise noted)	Feedstuff[1]				
	Alfalfa hay	Alfalfa haylage	Brome hay	Corn silage	DDGS[2]
Experiment 1					
DM, %	84.3	34.3	87.7	34.5	90.5
CP	20.6	15.8	8.5	8.6	28.3
ADF	32.1	37.4	44.4	24.8	10.6
NDF	37.0	44.6	69.6	39.6	31.3
Lignin	7.1	6.2	6.5	3.1	2.9
Starch	2.2	3.0	2.3	34.5	5.5
Ether extract	2.2	2.6	1.8	3.0	11.9
Ash	10.1	17.9	9.7	5.0	5.5
Ca	1.49	1.30	0.33	0.23	0.03
P	0.29	0.37	0.27	0.26	0.94
Mg	0.23	0.38	0.11	0.16	0.35
K	3.16	5.20	2.33	1.23	1.35
S	0.21	0.27	0.13	0.13	0.99
Na	0.02	0.03	0.01	0.01	0.23
Cl	0.17	0.26	0.63	0.17	0.17
Experiment 2					
DM, %	88.8	34.3	88.6	34.2	90.7
CP	19.3	15.6	8.8	7.6	28.3
ADF	34.5	38.4	42.5	30.0	10.8
NDF	43.6	45.9	67.2	43.5	34.7
Lignin	8.2	6.2	5.4	3.9	3.1
Starch	2.7	2.8	1.3	32.0	5.1
Ether extract	1.9	3.2	2.3	3.5	11.0
Ash	9.6	18.5	9.1	7.6	5.1
Ca	1.23	1.16	0.34	0.34	0.03
P	0.29	0.32	0.26	0.27	0.96
Mg	0.22	0.36	0.11	0.11	0.34
K	2.90	4.61	2.14	1.48	1.21
S	0.28	0.25	0.13	0.16	1.02
Na	0.08	0.04	0.02	0.02	0.17
Cl	0.12	0.25	0.49	0.29	0.11

[1]Mean of 4 periods is presented. Values determined by Cumberland Valley Analytical Services, Hagerstown, MD.

[2]Corn dried distillers grains with solubles (DDGS; POET Nutrition Inc., Sioux Falls, SD).

Table 3. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on TMR particle size distribution[1]

Particle size	Dietary treatment,[2] % retained (as-fed basis)				
	CONT	OL	STR	COMBO	SEM[3]
Experiment 1					
>19.0 mm	8.0	6.5	8.5	8.0	0.96
19.0–8.0 mm	25.5	26.5	26.0	28.7	0.86
8.0–1.18 mm	31.2	31.5	29.5	29.2	0.72
<1.18 mm	36.0	36.5	37.0	35.0	1.11
Experiment 2					
>19.0 mm	5.5	6.0	5.7	9.5	0.79
19.0–8.0 mm	26.5	25.7	26.7	28.2	0.84
8.0–1.18 mm	33.0	31.2	32.0	29.5	0.49
1.18 mm	36.0	37.5	36.5	33.5	0.95

[1]Measured using the Penn State Forage Particle Separator (Nasco, Fort Atkinson, WI).

[2]CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM; Exp. 1) and 8.7% (DM; Exp. 2) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% (DM; experiment 1) and 7.8% (DM; experiment 2) ground corn.

[3]Highest standard error of treatment mean is shown.

Table 4. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on DMI, milk yield, and composition

	Dietary treatment[1]					
Item	CON	OL	STR	COMBO	SEM[2]	*P*-value[3]
Experiment 1						
DMI, kg/d	25.2[a]	25.5[a]	24.1[ab]	22.0[b]	1.24	<0.01
Milk yield, kg/d	35.2	34.9	32.5	30.4	3.57	0.06
3.5% FCM[4]	32.6[a]	30.6[ab]	28.4[b]	24.4[c]	3.40	<0.01
Fat, %	3.19[a]	2.75[b]	2.88[ab]	2.21[c]	0.18	<0.01
Fat yield, kg/d	1.10[a]	0.97[ab]	0.91[b]	0.69[c]	0.12	<0.01
Protein, %	2.89	2.92	3.00	2.94	0.08	0.41
Protein yield, kg/d	0.99	1.00	0.95	0.90	0.12	0.29
MUN, mg/dL	14.1	13.5	13.9	13.5	1.02	0.53
BW, kg	700	708	702	698	30.9	0.51
BCS[5]	3.3	3.3	3.3	3.25	0.13	0.45
Experiment 2						
DMI, kg/d	22.2	22.2	22.9	21.7	0.63	0.46
Milk yield, kg/d	27.1	26.2	27.0	26.2	1.01	0.69
3.5% FCM	26.5[a]	23.6[b]	24.2[b]	22.8[b]	1.12	<0.01
Fat, %	3.35[a]	3.00[b]	3.03[b]	2.73[c]	0.15	<0.01
Fat yield, kg/d	0.90[a]	0.77[b]	0.78[b]	0.71[b]	0.05	<0.01
Protein, %	3.12	3.14	3.22	3.22	0.05	0.08
Protein yield, kg/d	0.85	0.81	0.85	0.84	0.03	0.56
MUN, mg/dL	11.1[a]	11.6[a]	11.3[a]	10.7[b]	0.38	0.01
BW, kg	637	642	644	642	12.7	0.35
BCS	3.4	3.4	3.4	3.4	0.07	0.97

[a–c]Means in the same row with different superscript differ ($P < 0.05$).

[1]CON = control diet; OL = similar to control diet with additional corn oil; STR = similar to control diet with additional ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of corn oil and ground corn.

[2]Highest standard error of treatment mean is shown.

[3]Main effect of treatment.

[4]Calculated as [milk fat (kg) × 16.218] + [milk yield (kg) × 0.4324].

[5]On a 1–5 scale (Wildman et al., 1982).

resulted in lower ($P < 0.01$) MUN, 10.7 ± 0.38 mg/dL. Milk fat concentration was affected by treatment ($P < 0.01$); values were 3.35, 3.00, 3.03, and 2.73 ± 0.15% for CON, OL, STR, and COMBO, respectively. Fat yield of the CON diet was 0.90 kg/d; this value was greater ($P < 0.01$) compared with 0.77, 0.78, and 0.71 ± 0.05 for OL, STR, and COMBO, respectively. Concomitant with these observations, FCM was reduced ($P < 0.01$) from 26.5 ± 1.12 kg/d with the CON diet to 23.6, 24.2, and 22.8 ± 1.12 kg/d by cows consuming the OL, STR, and COMBO diets, respectively. Body weight and BCS were not affected by treatment in either experiment.

Ruminal pH, Concentration of VFA, and Ammonia

Ruminal measurements are presented in Table 7 and diurnal pattern of ruminal pH is depicted in Figure 1. Concentration of ammonia in rumen fluid was similar across treatments, with an average of 13.8 ± 2.47 mg/dL. Similarly, no significant effect of treatment was observed on ruminal pH, averaging 5.87 ± 0.08 with an average minimum of 5.50 ± 0.05 and an average maximum of 6.71 ± 0.14 across treatments. Total concentrations of VFA were similar among all treatments, averaging 119 ± 3.43 m*M*. The proportion of acetate for the OL, STR, and COMBO diets was similar, at 62.6, 61.8, and 60.7 ± 1.34 mol/100 mol, respectively. These values tended to be lower ($P = 0.08$) compared with the CON treatment, which resulted in 64.3 ± 1.34 mol/100 mol. Conversely, molar proportion of propionate tended to increase ($P = 0.07$) from 22.3 for the CON treatment to 24.3, 25.4, and 25.8 ± 1.51 mol/100 mol for OL, STR, and COMBO, in that order. There was a trend ($P = 0.09$) for reducing the ratio of acetate to propionate with the addition of corn oil and ground corn, the ratio was 2.91, 2.60, 2.55, and 2.37 for CON, OL, STR, and COMBO, respectively.

Nutrient Digestibility

Estimates of apparent total-tract digestibility were similar across treatments for DM ($P = 0.57$) and OM ($P = 0.52$), averaging 69.1 ± 3.43 and 71.0 ± 3.14%, respectively. Apparent digestibility of CP averaged 72.4 ± 4.04% for all treatments ($P = 0.67$). There were no effects of diet on apparent digestibility of NDF; estimates were 52.0, 56.5, 43.7, and 46.6 ± 5.36% for the CON, OL, STR, and COMBO diets, respectively.

Table 5. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on milk FA concentration

	Dietary treatment[1]					
FA, g/100 g of FA	CON	OL	STR	COMBO	SEM[2]	*P*-value[3]
4:0	4.42[a]	4.01[a]	4.10[a]	3.26[b]	0.21	<0.01
6:0	2.18[a]	1.72[bc]	1.90[ab]	1.36[c]	0.12	<0.01
8:0	1.17[a]	0.84[bc]	0.97[ab]	0.65[c]	0.08	0.01
10:0	2.51[a]	1.79[bc]	2.09[ab]	1.42[c]	0.19	0.02
11:0	0.04[a]	0.03[ab]	0.04[ab]	0.02[b]	0.008	0.11
12:0	2.82[a]	2.17[bc]	2.48[ab]	1.90[c]	0.18	0.02
13:0	0.09[a]	0.08[ab]	0.09[bc]	0.07[c]	0.007	0.03
iso 14:0	0.13[a]	0.11[bc]	0.12[ab]	0.10[c]	0.009	0.02
14:0	9.56[a]	7.93[b]	8.82[ab]	7.42[b]	0.55	0.05
iso 15:0	0.22[a]	0.19[b]	0.18[bc]	0.15[c]	0.009	<0.01
ante 15:0	0.53[a]	0.48[b]	0.45[b]	0.41[c]	0.02	<0.01
14:1	0.74	0.71	0.84	0.87	0.07	0.35
15:0	0.86[a]	0.80[ab]	0.81[ab]	0.78[b]	0.05	0.15
iso 16:0	0.26	0.22	0.24	0.21	0.02	0.28
16:0	23.24[a]	21.43[b]	22.10[ab]	21.85[b]	0.78	0.07
iso 17:0	0.33	0.34	0.31	0.32	0.02	0.48
16:1	0.89[b]	0.93[b]	1.03b	1.39a	0.10	<0.01
ante 17:0	0.43[a]	0.37[b]	0.35bc	0.31c	0.03	<0.01
17:0	0.46	0.45	0.43	0.43	0.02	0.39
18:0	13.0	13.95	13.17	13.06	0.65	0.68
trans-4 18:1	0.027[b]	0.033[a]	0.027[b]	0.028[b]	0.002	0.03
trans-5 18:1	0.02[b]	0.03[a]	0.02[b]	0.03[ab]	0.002	0.04
trans-6–8 18:1	0.44[b]	0.63[a]	0.55[ab]	0.67[a]	0.06	0.02
trans-9 18:1	0.33[b]	0.43[a]	0.38[ab]	0.42[a]	0.03	0.10
trans-10 18:1	0.62[b]	1.92[b]	1.58[b]	3.44[a]	0.54	0.01
trans-11 18:1	1.70	2.08	1.82	1.25	0.33	0.36
trans-12 18:1	0.66[b]	0.77[a]	0.66[b]	0.72[ab]	0.05	0.08
cis-9 18:1	21.61[c]	24.50[ab]	23.80[bc]	26.69[a]	1.23	0.01
cis-11 18:1	0.47[b]	0.52[b]	0.57[b]	0.70[a]	0.07	0.01
C18:2 n-6	4.00	4.24	4.02	4.29	0.27	0.58
20:0	0.14	0.14	0.14	0.14	0.007	0.85
C18:3 n-3	0.35[a]	0.31[b]	0.30[bc]	0.27[c]	0.02	<0.01
18:2 *cis*-9,*trans*-11	0.84	1.10	0.98	0.80	0.17	0.47
18:2 *trans*-10,*cis*-12	0.00[b]	0.00[b]	0.008[ab]	0.024[a]	0.006	0.05
20:2	0.04	0.04	0.04	0.04	0.002	0.61
22:0	0.05[a]	0.04[b]	0.05[ab]	0.04[b]	0.003	0.06
20:3 n-6	0.15[a]	0.13[ab]	0.14[ab]	0.12[b]	0.01	0.11
20:4 n-6	0.19[a]	0.15[bc]	0.17[ab]	0.14[c]	0.007	<0.01
Unknown	4.44	4.37	4.23	4.18	0.16	0.35
<16C[4]	25.3[a]	20.9[bc]	22.9[ab]	18.4[c]	1.21	0.02
16C	23.5[a]	21.6[b]	22.3[ab]	22.1[b]	0.78	0.06
>16C	51.2[b]	57.5[a]	54.8[ab]	59.5[a]	1.91	0.02

[a–c]Means in the same row with different superscript differ ($P < 0.05$).

[1]CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% ground corn.

[2]Highest standard error of treatment mean is shown.

[3]Main effect of treatment.

[4]Fatty acids <16C originate from de novo synthesis, those >16C originate from uptake from plasma, and 16C originate from both sources.

Digestibility of starch was similar ($P = 0.76$) across treatments, averaging 98.7 ± 0.15%.

DISCUSSION

Research by Janicek et al. (2008) and Ranathunga et al. (2010) has shown that DDGS may be effectively fed to dairy cows at 20% of dietary DM. Nonetheless, this feedstuff is usually included at much lower levels (Hollmann et al., 2011) because the high content of fat in the feedstuff is perceived as a cause of MFD. This may be related to altered ruminal fermentation depending on the level, source, and degree of SFA (Onetti et al., 2001). We hypothesized that feeding DDGS in a TMR with high starch content may predispose cows to undergo MFD due to increased ruminal fermentation

Table 6. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on milk FA production

	Dietary treatment[1]					
FA, g/d	CON	OL	STR	COMBO	SEM[2]	*P*-value[3]
4:0	45.1[a]	36.3[a]	35.7[a]	21.7[b]	4.86	<0.01
6:0	22.3[a]	15.9[b]	16.9[ab]	9.0[c]	2.70	<0.01
8:0	11.9[a]	7.8[b]	8.7[ab]	4.3[c]	1.51	<0.01
10:0	25.7[a]	16.7[b]	18.8[ab]	9.3[c]	3.43	<0.01
11:0	0.44[a]	0.26[ab]	0.32[a]	0.11[b]	0.08	0.04
12:0	28.9[a]	20.1[b]	21.9[ab]	12.2[c]	3.65	<0.01
13:0	0.95[a]	0.71[b]	0.72[b]	0.46[c]	0.10	0.01
iso 4:0	1.32[a]	0.93[b]	1.07[b]	0.67[c]	0.16	<0.01
14:0	98.1[a]	74.0[b]	76.8[ab]	47.9[c]	12.70	<0.01
iso 15:0	2.29[a]	1.71[b]	1.55[b]	1.01[c]	0.23	<0.01
ante 15:0	5.47[a]	4.27[b]	3.90[b]	2.65[c]	0.57	<0.01
14:1	7.58[a]	6.77[ab]	6.98[ab]	5.46[b]	1.18	0.12
15:0	8.81[a]	7.22[b]	6.66[b]	4.87[c]	0.77	<0.01
iso 16:0	2.64[a]	1.93[b]	2.15[b]	1.45[c]	0.35	<0.01
16:0	238.1[a]	196.3[ab]	189.9[ab]	140.8[c]	30.4	<0.01
iso 17:0	3.33[a]	2.97[a]	2.56[b]	2.07[c]	0.27	<0.01
16:1	9.05	8.24	8.33	8.41	0.88	0.59
ante 17:0	4.38[a]	3.35[b]	3.02[b]	2.08[c]	0.50	<0.01
17:0	4.74[a]	3.96[b]	3.59[b]	2.80[c]	0.44	<0.01
18:0	132.9[a]	121.9[a]	111.8[a]	84.91[b]	13.75	0.01
trans-4 18:1	0.28[ab]	0.30[a]	0.24[bc]	0.19[c]	0.04	0.02
trans-5 18:1	0.26[ab]	0.28[a]	0.21[bc]	0.19[c]	0.03	0.04
trans-6–8 18:1	4.46[b]	5.52[a]	4.46[b]	4.18[b]	0.53	0.01
trans-9 18:1	3.37[ab]	3.84[a]	3.16[bc]	2.64[c]	0.41	0.02
trans-10 18:1	6.31[c]	15.72[ab]	10.86[bc]	20.82[a]	3.29	0.04
trans-11 18:1	17.54	18.96	16.48	8.82	3.82	0.21
trans-12 18:1	6.77[a]	6.86[a]	5.73[ab]	4.60[b]	0.84	0.01
cis-9 18:1	220.2[a]	217.9[a]	198.4[ab]	172.1b	24.98	0.03
cis-11 18:1	4.73	4.55	4.43	4.30	0.43	0.74
C18:2 n-6	40.81	37.29	34.64	27.52	4.62	0.05
20:0	1.47[a]	1.24[ab]	1.19[b]	0.86[c]	0.13	<0.01
C18:3 n-3	3.55[a]	2.75[b]	2.66[b]	1.78[c]	0.34	<0.01
18:2 *cis*-9,*trans*-11	8.67	10.24	8.66	5.46	2.06	0.28
18:2 *trans*-10,*cis*-12	0	0	0.04	0.14	0.04	0.13
20:2	0.42[a]	0.37[a]	0.34[ab]	0.26[b]	0.06	0.02
22:0	0.55[a]	0.39[b]	0.40[b]	0.25[c]	0.06	<0.01
20:3 n-6	1.57[a]	1.19[ab]	1.15[ab]	0.77[b]	0.21	0.02
20:4 n-6	1.95[a]	1.33[bc]	1.45[b]	0.87[c]	0.21	0.01
Unknown	45.34[a]	39.05[ab]	36.56[b]	26.63[c]	4.96	<0.01
Total	1,023.13[a]	899.22[ab]	852.40[b]	644.62[c]	113.1	<0.01
<16C[4]	259.01[a]	192.71[b]	200.04[ab]	119.67[c]	31.32	<0.01
16C	241.46[a]	198.23[b]	192.03[b]	142.27[c]	30.62	<0.01
>16C	522.66[a]	508.27[a]	460.33[a]	382.69[b]	54.16	0.01

[a–c]Means in the same row with different superscript differ ($P < 0.05$).

[1]CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% ground corn.

[2]Highest standard error of treatment mean is shown.

[3]Main effect of treatment.

[4]Fatty acids <16C originate from de novo synthesis, those >16C originate from uptake from plasma, and 16C originate from both sources.

and that the addition of corn oil would exacerbate this response by altering biohydrogenation of PUFA.

Nutrient content of feeds were within expected values; however, the method of drying may have affected N volatilization, which may result in underestimation of CP. When N volatilization is of concern, an alternative to overcome such a problem would be to lyophilize samples. In experiment 1, DMI was similar for the CON, STR, and OL diets. However, we observed a 3-kg reduction in DMI when cows consumed the COMBO treatment, which had greater concentrations of starch and fat. It is possible that feeding DDGS and supplemental fat decreased DMI by increasing intake of PUFA. For example, Abdelqader et al. (2009) re-

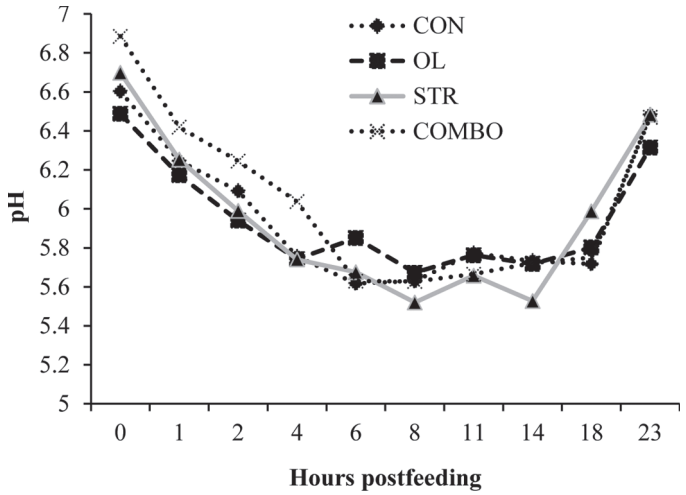

Figure 1. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on diurnal pattern of ruminal pH. SEM = 0.09. $P = 0.70$ for treatment and 0.17 for treatment × time. CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% ground corn.

ported that diets containing DDGS resulted in greater intake of PUFA; similarly, Harvatine and Allen (2006) reported reductions in DMI due to PUFA intake. Additionally, Harvatine et al. (2009) suggested that cows under MFD may experience an energy-sparing effect partitioning energy toward body reserve replenishment as a result of reduced milk energy output. Furthermore, Bradford et al. (2008) reported that when cows consumed diets rich in PUFA an increase in circulating cholecystokinin and glucagon-like peptide-1 was observed, both of which have hypophagic effects. Janicek et al. (2008) and Benchaar et al. (2013) reported that increased DMI was paralleled by increased milk yield when cows consumed DDGS. Janicek et al. (2008) attributed changes in DMI to a greater proportion of particles <1.18 mm, which may have reduced rumen fill and allowed for greater intake. This does not seem to be the case in our experiments, as the changes in the ratio of forage to concentrate did not affect particle size distribution; furthermore, DMI was not affected in experiment 2. Rather than a physical response, the reasons for these inconsistencies between experiments 1 and 2 may be related to energy requirements driven by milk production. Overall, DMI and milk production were lower during the second experiment, and this may suggest lower energy needs by those cows, hence lower DMI.

Diet-induced MFD is characterized by a high specificity in the downregulation of enzymes for synthesis of milk fat and no other milk components are affected (Peterson et al., 2003). The changes in *trans* isomers and de novo and preformed FA observed in our study were consistent with classical diet-induced MFD (Bauman and Griinari, 2003). We hypothesized that adding

Table 7. Effects of adding corn oil, starch, or a combination of both to a diet containing dried distillers grains with solubles (DDGS) on ruminal pH, VFA, and ammonia concentrations

	Dietary treatment[1]					
Item	CON	OL	STR	COMBO	SEM[2]	*P*-value[3]
Rumen pH						
Minimum	5.52	5.59	5.41	5.47	0.05	0.15
Maximum	6.68	6.55	6.73	6.88	0.14	0.24
Mean[4]	5.86	5.86	5.86	5.90	0.08	0.90
Rumen ammonia,[4] mg/dL	12.7	15.2	14.6	12.8	2.47	0.57
Total VFA,[4] m*M*	122[a]	119[ab]	121[ab]	115[b]	3.43	0.13
VFA,[4] mol/100 mol						
Acetate	64.3[a]	62.6[ab]	61.8[ab]	60.7[b]	1.34	0.08
Propionate	22.3[b]	24.3[ab]	25.4[a]	25.8[a]	1.51	0.07
Butyrate	10.7	10.5	10.1	10.4	0.45	0.63
Valerate	1.52	1.52	1.54	1.66	0.07	0.38
Isovalerate	0.47[b]	0.48[b]	0.51[b]	0.61[a]	0.03	0.02
Isobutyric	0.64[b]	0.59[b]	0.63[b]	0.73[a]	0.04	0.03
Acetate:propionate	2.91[a]	2.60[ab]	2.55[ab]	2.37[b]	0.23	0.09

[a,b]Means in the same row with different superscript differ ($P < 0.05$).

[1]CON = control diet; OL = similar to control diet with additional 0.97% (DM) corn oil; STR = similar to control diet with additional 8.5% (DM) ground corn to increase starch content; COMBO = diet similar to control but containing an additional combination of 0.97% corn oil and 7.6% ground corn.

[2]Highest standard error of treatment mean is shown.

[3]Main effect of treatment.

[4]Weighted means determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972).

fat and starch would add more PUFA and lower rumen pH, respectively, resulting in conditions leading to the formation of CLA isomers that downregulate milk fat synthesis in the mammary gland (Perfield et al., 2007; Harvatine et al., 2009). It may be useful to note that fat concentration in milk from cows consuming the CON diet was unexpectedly below the herd average. In spite of this, we were successful at inducing MFD. When compared with the CON diet, the differences in FCM among OL, STR, and COMBO diets suggest negative additive effects. This response is due to a decrease in milk fat concentration that dropped 12% when cows consumed either the OL or the STR treatments, and 30% when the COMBO treatment was fed relative to the control. These reductions resulted in 15 and 37% lower milk fat yield for the same diets relative to the control treatment. Based on rumen pH and ammonia concentration, no evidence of altered rumen environment among treatments was seen, therefore suggesting that altered biohydrogenation leading to MFD may develop without evident modifications in rumen conditions. Such a scenario has been reported by Zened et al. (2013), who fed dry cows a combination of high sunflower oil and high starch (7.3% crude fat and 33% starch, dietary DM). They did not observe a significant interaction of these factors on rumen pH but reported a significant increased formation of *trans*-10 isomers in rumen fluid. This type of isomer is associated with *trans*-10,*cis*-12 CLA, a known potent inhibitor of milk fat synthesis (Baumgard et al., 2000). This particular FA was detected in the milk in 3 out of 4 cows consuming the COMBO diet and only in 1 cow consuming the STR diet, whereas milk from all the remaining cows did not contain detectable levels of this FA. *Trans*-10 C18:1 is a major intermediate of the same alternate biohydrogenation pathway and the concentration and yield of this FA increased more than 2-fold with the STR and OL and more than 3-fold with COMBO. These observations indicate alterations in the ruminal biohydrogenation pathways and may have had an effect on mammary synthesis of FA. The mammary gland obtains FA <16 carbons for milk fat from de-novo synthesis and FA >16 carbons from the bloodstream; our data indicate a reduction in both pathways and that de-novo synthesis was affected to a greater extent when cows consumed the COMBO diet. These cows produced less than 50% short-chain FA compared with the control diet, whereas production of long-chain FA with the COMBO diet was 73% that of the control diet. This is consistent with previous observations that de novo FA synthesis is decreased during MFD (Loor and Herbein, 1998) and the reduction is less severe for preformed FA incorporation (Baumgard et al., 2001). Furthermore, we observed changes in concentration and yield of *iso*- FA in milk from cows under severe MFD. These FA may be used to characterize ruminal bacteria (Ifkovitz and Ragheb, 1968; Kaneda, 1991). Therefore, we suggest that these changes may be an indicator of shifts in ruminal bacterial populations during MFD, as reported by Weimer et al.(2010) and Mohammed et al. (2012). Overall, our data suggest that MFD may develop in diets that contain DDGS inclusion levels ≥20% of dietary DM and may occur if starch or ether extract are ≥20 and 6%, respectively.

CONCLUSIONS

The addition of corn to increase the starch content of the diets to induce low rumen pH was not successful, which suggests that low rumen pH is not an essential condition for the development of MFD. In addition, the increase in intake of PUFA alone may spur the onset of the disorder when diets contain a high inclusion of DDGS. Milk FA profile was altered by inclusion of corn oil and starch, and the combination of these factors resulted in greater concentration and yield of *trans*-10,*cis*-12 CLA and *trans*-10 18:1. Further research is needed to relate alterations in concentration and yield of *iso* FA in milk with changes in ruminal microbial populations.

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