Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk

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ABSTRACT

Previous work has indicated that dietary palmitic acid (C16:0) may increase milk fat yield. The effect of a dietary C16:0-enriched fat supplement on feed intake, yield of milk and milk components, and feed efficiency was evaluated in an experiment with a cross-over arrangement of treatments with 25-d periods. A fermentable starch challenge on the last 4 d of each period was utilized as a split-plot within period. Sixteen mid-lactation Holstein cows (249 ± 33 d in milk) were assigned randomly to treatment sequence. Treatments were either a C16:0-enriched (~85% C16:0) fat supplement (fatty acid treatment, FAT, 2% dry matter) or a control diet (CON) containing no supplemental fat. Diets containing dry ground corn grain were fed from d 1 through 21 of each period. On the last 4 d of each period, dry ground corn was replaced by high-moisture corn grain on an equivalent dry matter basis to provide a fermentable starch challenge. Response variables were averaged for d 18 to 21 (immediately before the fermentable starch challenge) and d 22 to 25 (during the fermentable starch challenge). We observed no treatment effects on milk yield or milk protein yield. The FAT treatment increased milk fat concentration from 3.88 to 4.16% and fat yield from 1.23 to 1.32 kg/d compared with CON. The FAT treatment decreased dry matter intake by 1.4 kg/d and increased conversion of feed to milk (3.5% fat-corrected milk yield/dry matter intake) by 8.6% compared with CON. The increase in milk fat yield by FAT was entirely accounted for by a 27% increase in 16-carbon fatty acid output into milk. Yields of de novo and preformed fatty acids were not affected by FAT relative to CON. The fermentable starch challenge did not affect milk fat concentration or yield. Results demonstrate the potential for a dietary C16:0-enriched fat supplement to improve milk fat concentration and yield as well as efficiency of conversion of feed to milk. Further studies are required to verify and extend these results and to determine whether re-

sponses are similar across different diets and levels of milk production.

Key words: dairy cow, fat supplementation, milk fat, palmitic acid

INTRODUCTION

The concentration and yield of milk fat are affected by the nutrition of the dairy cow, and diets that allow for an improvement in milk fat output may be advantageous economically. Fat supplements are commonly fed to increase dietary energy density and support milk production. The productive responses, however, may vary depending on dietary factors such as the FA profile and the form of fat being fed. There is a growing interest in understanding the effects of different fat sources on milk production parameters, and special attention has been given to determining the effects of specific SFA, particularly palmitic (C16:0) and stearic (C18:0) acids (e.g., Enjalbert et al., 2000; Piantoni et al., 2013).

Milk fat concentration and yield are often increased when SFA supplements are fed. Relling and Reynolds (2007) compared the feeding of an SFA supplement (free FA) with Ca-salts of palm FA distillate and Ca-salts of soybean FA and reported that milk fat concentration decreased as degree of unsaturation increased. The SFA treatment also increased milk fat concentration and yield compared with the control diet (no fat supplement). There is, however, limited evidence to indicate that specific SFA are more or less effective at increasing milk fat. It has been reported that C16:0 when fed (Steele and Moore, 1968b; Steele, 1969) or infused into the duodenum (Enjalbert et al., 2000) increased milk fat yield compared with no fat supplementation or supplementation with C18:0.

Over the last decade, significant advances have contributed to our understanding of the interrelationship between rumen lipid metabolism and milk fat synthesis. Available evidence indicates that milk fat depression (MFD) is caused by shifts in rumen biohydrogenation of unsaturated FA and the passage of specific FA out of the rumen which, when absorbed, result in a decreased expression of lipogenic enzymes and a reduction in milk fat synthesis in the mammary gland (Bauman et al.,

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2011). Risk factors involved in MFD in dairy cows include decreased ruminal pH and a high concentration of unsaturated FA in the diet (Bauman et al., 2011). The inclusion of a highly fermentable starch source is also considered a risk factor for MFD (Bradford and Allen, 2004; Longuski et al., 2009); however, the extent of its effects has not been characterized yet in combination with fat supplements that may increase milk fat.

Based on the aforementioned results, we hypothesized that a C16:0-enriched fat supplement would increase milk fat yield and reduce the negative effects of a dietary-induced MFD challenge. Our primary objective was to compare the effect of a diet containing a C16:0-enriched fat supplement to a non-fat-supplemented control diet on feed intake, yields of milk and milk components, feed efficiency, and milk FA. Our secondary objective was to determine whether a C16:0-enriched fat supplement would mask the negative effects of a dietary-induced MFD challenge by utilizing a fermentable starch challenge (FSC) on the last 4 d of each period.

MATERIALS AND METHODS

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Sixteen mid-lactation Holstein cows (9 primiparous and 7 multiparous; 249 ± 33 DIM; mean $\pm$ SD) from the Michigan State University Dairy Field Laboratory (East Lansing) were randomly assigned to treatment sequence in a crossover design experiment with 25-d periods. Treatments consisted of a C16:0-enriched fat supplement (BergaFat F100, Berg+Schmidt, Hanover, Germany; fatty acid treatment, **FAT**) or a control diet containing no supplemental fat (**CON**). The C16:0-enriched fat supplement was a free FA product derived from palm oil and contained approximately 85% C16:0 and 90% total SFA, with the remainder being predominantly *cis*-9 C18:1 (Table 1). The supplement was included in the diet at 2% DM instead of soyhulls in CON (Table 2).

On the last 4 d of each period (d 22 to 25), an FSC was utilized as a split plot. Diets containing dry ground corn grain (**DC**) were fed from d 1 through 21 of each period. For the FSC, on the last 4 d of each period, the DC in the ration was replaced by high-moisture corn grain (**HMC**) on an equivalent DM basis. Response variables were averaged for d 18 to 21 (immediately before the FSC) and d 22 to 25 (during the FSC). Before the first treatment period, all cows were fed a common diet for 14 d to obtain baseline values for DMI, milk yield, and milk composition. At the beginning of the

experiment, BW of cows was 657 ± 45 kg and milk yield was 33.1 ± 3.3 kg/d (mean $\pm$ SD).

Corn silage was the major forage component, with corn grain (DC or HMC) as the major concentrate component of the diet (Table 2). The diets containing HMC were intended to be highly fermentable to ensure detection of treatment effects during the FSC at the end of each period. Diets were formulated to contain 22% forage NDF and 17% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. The concentration of DM was determined twice weekly for forages and diets were adjusted when necessary.

Data and Sample Collection

Throughout the experiment, cows were housed in individual tiestalls. Access to feed was blocked from 0800 to 1000 h to allow for collection of orts and offering feed. Cows were fed 115% of expected intake at 1000 h daily. Diet ingredients and orts were sampled daily during d 18 to 25 and composited separately for the DC and HMC diets during each period. Water was available ad libitum in each stall, and stalls were bedded with sawdust and cleaned twice daily.

Cows were milked twice daily at 0400 and 1500 h, with milk yield recorded electronically (Boumatic ProVantage Dairy Management System, Boumatic, Madison, WI) at each milking throughout the experiment. Milk was sampled at each milking from d 18 to 25. Two samples were taken from each cow at each milking; one aliquot was collected in a sealed tube with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at -20°C until analyzed for FA composition. Body weight was measured on the last 2 d of the preliminary period as well as on d 20, 21, 24, and 25 of each period. Three trained investigators determined BCS on a 5-point scale on the last day of the preliminary period and on d 21 and 25 of each period (Wildman et al., 1982).

Table 1. Fatty acid composition (g/100 g of total FA) of the C16:0-enriched fat supplement¹

FA	g/100 g of total FA
12:0	0.11
14:0	2.61
16:0	86.1
16:1 <i>cis</i> -9	0.14
18:0	1.06
18:1 <i>cis</i> -9	8.24
18:2 <i>cis</i> -9, <i>cis</i> -12	1.47

¹Values are means of 4 replicate GLC analyses.

Table 2. Ingredient and nutrient composition of soyhulls (CON) and C16:0-enriched fat supplement (fatty acid treatment, FAT) diets fed during the treatment periods

Item	CON		FAT	
Diet ingredient, % of DM				
Corn silage	32.0		32.0	
Corn ¹	16.0		16.0	
Soybean meal	13.8		13.8	
Alfalfa silage	9.1		9.1	
Vitamin and mineral mix ²	8.5		8.5	
Soyhulls	9.2		7.2	
Cottonseed with lint	3.9		3.9	
Alfalfa hay	3.8		3.8	
Grass hay	3.7		3.7	
C16:0-enriched fat supplement ³	—		2.0	
Nutrient composition	DC ⁴	HMC ⁵	DC	HMC
DM, %	52.1	50.0	52.2	50.1
NDF, % of DM	33.9	32.3	32.5	30.8
Forage NDF, % of DM	22.2	20.4	22.2	20.4
Starch, % of DM	29.2	27.8	29.2	27.8
CP, % of DM	15.0	16.6	14.8	16.4
Total FA, % of DM	2.8	2.7	4.8	4.7
FA concentration, g/100 g of total FA				
14:0	0.6	0.7	1.4	1.5
16:0	18.5	19.1	46.5	47.6
16:1 <i>cis</i> -9	0.7	0.8	0.5	0.5
18:0	3.4	3.5	2.4	2.4
18:1 <i>cis</i> -9	20.9	20.5	15.6	15.3
18:2 <i>cis</i> -9, <i>cis</i> -12	47.7	46.9	28.6	27.7
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	4.3	4.6	2.5	2.6
22:0	0.5	0.4	0.3	0.3
24:0	0.4	0.4	0.2	0.2
Σ Others	3.0	3.1	2.0	1.9
Σ SFA	23.7	24.4	51.1	52.3
Σ MUFA	23.5	23.1	17.2	16.9
Σ PUFA	52.8	52.5	31.6	30.8

¹Dry ground corn grain (DC; d 1 to 21 of each period) and high-moisture corn grain (HMC; d 22 to 25 of each period).

²Vitamin and mineral mix contained 58.3% dry ground shell corn, 12.3% limestone, 7.9% sodium bicarbonate, 6.8% dicalcium phosphate, 3.2% urea, 3.2% magnesium, 3.2% tallow, 3.0% white salt, and <1% of each of the following; trace minerals, biotin, vitamin E, vitamin A, vitamin D, and selenium.

³BergaFat F100, Berg+Schmidt, Hamburg, Germany.

⁴Dry ground corn grain treatment (d 1 to 21 of each period).

⁵High-moisture corn grain treatment (d 22 to 25 of each period).

Sample Analysis

Diet ingredients andorts were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1- to 2-mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients were analyzed for NDF, CP, and starch according to the methods described by Harvatine and Allen (2005). Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990; method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Lansing, MI). Yields of 3.5% FCM, ECM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period (Reist et al., 2002).

Milk samples used for analysis of FA composition were composited based on milk fat yield of individual samples taken at each milking during collection periods (d 18–21 and d 22–25). Composite samples (~40 mL) were centrifuged at $17,800 \times g$ for 30 min at 4°C, and the fat cake was collected. An adapted protocol of the extraction procedure developed by Hara and Radin (1978) using *n*-hexane/isopropanol (3:2, vol/vol) was used for the extraction of total lipids from the fat cake. Fatty acid methyl esters (FAME) were prepared by shaking a mixture of 2.5 mL of *n*-hexane containing 25 mg of lipids and 0.5 mL of 0.5 *M* sodium methoxide solution in methanol for 5 min. One gram of sodium bisulfate was added and the vial was mixed by vortexing. After a 5-min centrifugation at $6,000 \times g$, the supernatant containing the FAME was transferred into a 2-mL vial and used directly for GLC analysis.

Fatty acid composition covering approximately 70 FA in the range C4:0 to C24:0 was determined by a GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) with a split injector (1:100 split ratio) and a flame-ionization detector (**FID**) using a CP-Sil 88 WCOT (wall-coated open tubular) fused-silica column (100 m × 0.25 mm i.d. × 0.2-μm film thickness; Varian Inc., Lake Forest, CA). Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the FID at 40 mL/min. The other FID gases were purified air at 400 mL/min and nitrogen makeup gas at 30 mL/min. Injector and detector temperature was kept at 250°C. The oven program was: initial temperature of 40°C and held for 4 min, programmed at 13°C/min to 175°C and held for 27 min, then programmed at 4°C/min to 215°C and held for 35 min. Injection volume was 1 μL. Integration and quantification (GCsolution software version 2.32.00; Shimadzu) were based on FID response. Individual FAME were identified by comparison of retention times with known FAME standards (GLC reference standard 463, GLC reference standard 481-B, and conjugated octadecadienoic mixture #UC-59-M from Nu-Chek Prep Inc., Elysian, MN; Supelco 37 component FAME mix, *cis/trans* FAME mix, bacterial acid methyl ester mix, and PUFA No. 3 mix from Supelco Inc., Bellefonte, PA). Short-chain FAME were corrected for mass discrepancy using the response factors published by Ulberth and Schrammel (1995). Yields of individual FA (g/d) were calculated using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol and other milk lipid classes (Schauff et al., 1992; Glasser et al., 2007).

Fatty acid concentrations of feed ingredients were determined using a modification of the method developed by Sukhija and Palmquist (1988). Dried, ground samples were weighed to yield ~10 to 50 mg of FA, and *cis*-10 C17:1 (1:1 mg/mL acetone) was added as the internal standard. Samples were dissolved in toluene at twice the volume of the internal standard in acetone solution (2:1 mL/mL). A 5% methanolic sulfuric acid solution was added at two-thirds the sample volume and samples were incubated overnight at 50°C. Once at room temperature, samples were neutralized with twice their volume using a 5% aqueous sodium chloride solution and FAME extracted using *n*-hexane. The solvent layer was washed with a 6% aqueous potassium bicarbonate solution and dried over anhydrous sodium sulfate. The FAME were filtered through silica gel and charcoal, solvents removed under nitrogen flux at 37°C, the FAME weighed, and a 1% solution with *n*-hexane prepared on a weight basis, which was used for GLC analysis.

Feed FAME were determined on a GC-2010 Plus gas chromatograph (Shimadzu) equipped with a split injector (1:100 split ratio) and a FID using a CP8827 WCOT fused-silica column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; Varian Inc.). Carrier and FID gases were the same as described for milk FAME analyses. Injector and detector temperature was kept at 270°C. The oven program was as follows: initial temperature of 140°C and held for 1 min, programmed at 5°C/min to 225°C, then programmed at 50°C/min to 250°C held for 5.5 min. Injection volume was 1 μL. Individual FAME were identified by comparison of retention times with known FAME standards (GLC reference standard 63-A and GLC reference standard 455 from Nu-Chek Prep Inc.).

The gross efficiency of FA yield response (**FAYR**) to the C16:0-enriched supplement was calculated as the ratio between the additional FA secreted in milk (FAT minus CON) and the additional dietary FA consumed (FAT minus CON) when cows were fed DC for both total FA and 16-carbon FA (Piantoni et al., 2013).

Statistical Analysis

Our primary objective was to compare the effects of a diet containing a C16:0-enriched fat supplement to a non-fat-supplemented control diet on productive performance. For this comparison, effects of FAT during the 21-d DC periods were determined. The main effect of FAT was not determined across corn grain sources because cows only received HMC for 4 d as a FSC, thus not allowing for sufficient time for diet adaptation. Data during the DC period were analyzed statistically as a mixed model using the fit model procedure in JMP (version 10.0.0; SAS Institute Inc., Cary, NC). The model included the random effect of cow and the fixed effects of period, treatment, and the period by treatment interaction.

Our secondary objective was to determine whether a C16:0-enriched fat supplement would reduce the negative effects of a FSC. Data were analyzed statistically using the MIXED procedure of SAS (version 9.2; SAS Institute Inc.) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + CPT_{ijk} + D_l + TD_{kl} + e_{ijkl},$$

where μ = overall mean; C_i = random effect of cow ($i = 1$ to 16); P_j = fixed effect of period ($j = 1$ to 2); T_k = fixed effect of FAT ($k = 1$ to 2); CPT_{ijk} = interaction of cow, period, and FAT; D_l = fixed effect of FSC ($l = 1$ to 2); TD_{kl} = interaction of FAT and FSC; and e_{ijkl} =

Table 3. Milk production response to C16:0-enriched fat supplement and starch fermentability treatments¹

Item	CON		FAT		SE	P-value ²		
	DC ²	HMC ³	DC	HMC		FAT	CHO	FAT × CHO
DMI, kg/d	24.7	23.8	23.3	23.8	0.7	<0.01	0.48	0.02
Milk yield, kg/d								
Milk	32.0	32.0	32.0	32.2	1.5	0.99	0.95	0.80
3.5% FCM	33.6	33.7	35.1	35.5	1.4	0.10	0.54	0.63
ECM	34.0	34.0	35.1	35.4	1.3	0.24	0.56	0.62
Milk composition								
Fat, kg/d	1.23	1.23	1.32	1.33	0.05	<0.01	0.39	0.55
Fat, %	3.88	3.89	4.16	4.19	0.12	<0.001	0.51	0.71
Protein, kg/d	1.06	1.06	1.05	1.06	0.04	0.60	0.71	0.65
Protein, %	3.33	3.33	3.28	3.30	0.06	0.02	0.25	0.06
Milk lactose, kg/d	1.53	1.52	1.51	1.51	0.08	0.73	0.74	0.75
Lactose, %	4.75	4.73	4.71	4.68	0.07	0.10	<0.01	0.99
3.5% FCM/DMI, kg/kg	1.38	1.53	1.51	1.50	0.05	<0.001	0.40	0.05

¹Treatments were either control (CON; with 2% of diet DM as added soyhulls) or supplemental FA (fatty acid treatment, FAT; with 2% of diet DM as added C16:0-enriched fat supplement). Dry ground corn (DC) grain was fed d 1 to 21 and high-moisture corn (HMC) grain was fed d 22 to 25 of each period.

²FAT = main effect of fat supplementation for DC only (mean of d 18 to 21 of each period); CHO = main effect of fermentable starch challenge (mean of d 22 to 25 of each period); FAT × CHO = interaction of fat supplementation and fermentable starch challenge.

residual. The main effect of the FSC and its interaction with FAT were tested using residual mean square error.

Treatment effects were declared significant at $P \leq 0.05$ and trends for treatment effects at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and trends for interactions at $P \leq 0.15$. When interactions among main effects were significant, treatment means were separated using Tukey's Honestly Significant Difference test (SAS version 9.2; SAS Institute Inc.).

RESULTS

The FAT treatment increased total dietary FA concentration ~73% and C16:0 concentration ~150% compared with CON diets (Table 2). Concentrations of CP, forage NDF, and starch did not differ between FAT and CON diets (Table 2).

Compared with CON, FAT increased milk fat concentration by 7.2%, from 3.88 to 4.16% ($P < 0.001$), and fat yield by 7.3%, from 1.23 to 1.32 kg/d ($P < 0.01$; Table 3). Consequently, we observed a trend for FAT to increase 3.5% FCM yield ($P = 0.10$). The FAT treatment had no effect on yields of milk, milk protein, or milk lactose. The FSC resulted in no main effects or interactions on yields of milk, milk fat, milk protein, or milk lactose (Table 3). The FAT treatment decreased milk protein concentration slightly (1.6%; $P = 0.02$), and FSC increased milk protein concentration slightly for FAT only (interaction $P = 0.06$). The FSC ($P < 0.01$) decreased milk lactose concentration, and we observed a trend for FAT to also decrease lactose concentration ($P = 0.10$). Milk somatic cell numbers were not different across treatments (data not shown).

The FAT treatment decreased DMI 1.4 kg/d ($P < 0.01$) but had no effect on DMI during the FSC (interaction $P = 0.02$). Because of the increase in 3.5% FCM and the decrease in DMI, FAT resulted in an 8.6% improvement in the efficiency of conversion of feed to milk (3.5% FCM/DMI) compared with CON ($P < 0.001$; Table 3). The FSC increased conversion of feed to milk for CON only (interaction $P = 0.05$).

Concentrations of FA in milk and yields of selected FA are shown in Tables 4 and 5, respectively. The FAT treatment increased C16:0 concentration in milk 18.8% ($P < 0.001$), but concentrations of most other milk FA were lower for FAT compared with CON (Table 4). The FSC only had a minor effect on several FA, typically those present in very low concentrations in milk fat. The concentration of FA in milk fat according to source (<16 carbon FA from de novo synthesis in the mammary gland, >16 carbon FA originating from extraction from plasma, and 16-carbon FA originating from mixed sources) was altered by treatments. For CON and FAT, the FA profile of milk fat was 25.1 and 22.1 de novo FA (<16 carbon; $P = 0.002$), 36.5 and 43.3 16-carbon FA (C16:0 + *cis*-9 C16:1; $P < 0.001$), and 38.4 and 34.6 preformed FA (>16 carbon; $P < 0.001$) g/100 g of FA, respectively. Although we found no overall effect of FSC, we did observe a FAT × FSC interaction ($P = 0.05$), with a slight decrease in the concentration of 16-carbon FA in milk fat with HMC in CON but slightly higher 16-carbon FA concentration in milk with HMC in FAT. The FAT treatment increased total SFA 2.8% (70.3 to 72.3 g/100 g of FA, CON vs. FAT; $P < 0.01$) despite the large increase in C16:0. This was because of the reduction in the concentrations

Table 4. Milk FA concentration response to C16:0-enriched fat supplement and starch fermentability treatments¹

FA (g/100 g)	CON		FAT		SE	<i>P</i> -value ²		
	DC	HMC	DC	HMC		FAT	CHO	FAT × CHO
4:0	2.96	2.88	2.93	2.87	0.04	0.33	<0.01	0.54
6:0	1.95	1.90	1.80	1.69	0.04	0.02	<0.01	0.21
8:0	1.14	1.13	0.99	0.91	0.03	0.004	0.04	0.14
10:0	2.97	2.98	2.43	2.23	0.12	0.004	0.22	0.15
12:0	3.51	3.52	2.81	2.60	0.14	0.001	0.29	0.19
14:0	11.7	12.1	10.4	10.1	0.32	0.002	0.70	0.05
14:1 <i>cis</i> -9	0.84	0.81	0.77	0.71	0.04	0.002	<0.01	0.36
16:0	35.2	34.7	41.8	43.1	0.62	<0.001	0.50	0.05
16:1 <i>cis</i> -9	1.25	1.15	1.49	1.43	0.05	0.004	0.02	0.36
18:0	8.67	9.33	7.41	7.52	0.30	0.004	0.05	0.13
18:1 <i>trans</i> -4	0.01	0.01	0.01	0.01	0.00	0.02	0.08	0.59
18:1 <i>trans</i> -5	0.01	0.01	0.01	0.01	0.00	0.02	0.98	0.86
18:1 <i>trans</i> -6–8	0.19	0.19	0.17	0.17	0.00	<0.001	0.02	0.87
18:1 <i>trans</i> -9	0.17	0.18	0.15	0.15	0.01	0.01	0.10	0.32
18:1 <i>trans</i> -10	0.32	0.33	0.27	0.27	0.01	0.001	0.48	0.41
18:1 <i>trans</i> -11	0.64	0.66	0.52	0.54	0.03	0.001	0.20	0.66
18:1 <i>trans</i> -12	0.31	0.33	0.26	0.26	0.01	<0.001	0.08	0.37
18:1 <i>cis</i> -9	19.0	18.7	18.0	18.0	0.43	0.04	0.40	0.74
18:1 <i>cis</i> -11	0.55	0.57	0.49	0.51	0.02	0.003	0.02	0.73
18:2 <i>cis</i> -9, <i>cis</i> -12	2.11	2.00	1.89	1.77	0.05	0.008	<0.001	1.00
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.27	0.27	0.23	0.22	0.01	0.001	0.17	0.38
18:2 <i>cis</i> -9, <i>trans</i> -11	0.30	0.29	0.26	0.25	0.01	0.02	0.07	0.80
Others ³	5.90	5.93	4.89	4.75	0.10	<0.001	0.38	0.14
Summations ⁴								
De novo	25.1	25.4	22.1	21.1	0.61	0.002	0.34	0.10
Mixed	36.5	35.8	43.3	44.5	0.65	<0.001	0.63	0.05
Preformed	38.4	38.8	34.6	34.4	0.69	<0.001	0.68	0.21
Σ SFA	70.3	70.7	72.3	72.6	0.61	<0.01	0.14	0.95
Σ MUFA <i>cis</i>	22.7	22.4	21.7	21.5	0.50	0.08	0.26	0.79
Σ PUFA <i>cis</i>	2.79	2.67	2.46	2.33	0.06	0.001	<0.001	0.96

¹Treatments were either control (CON; with 2% of diet DM as added soyhulls) or supplemental FA (fatty acid treatment, FAT; with 2% of diet DM as added C16:0-enriched fat supplement). Dry ground corn (DC) grain was fed d 1 to 21 and high-moisture corn (HMC) grain was fed d 22 to 25 of each period.

²FAT = main effect of fat supplementation for DC only (mean of d 18 to 21 of each period); CHO = main effect of fermentable starch challenge (mean of d 22 to 25 of each period); FAT × CHO = interaction of fat supplementation and fermentable starch challenge.

³Consists of 44 identified FA ranging from C5:0 to C22:5 and less than 1.00 g/100 g of unidentified FA.

⁴De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originate from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

of C6:0 to *cis*-9 C14:1 (all $P < 0.05$). Total *cis* PUFA concentration decreased (2.8 to 2.5 g/100 g of FA, CON vs. FAT, $P = 0.001$), and we observed a trend for a decrease in total *cis* MUFA concentration (22.7 to 21.7 g/100 g of FA, CON vs. FAT; $P = 0.08$) in milk fat. Total *trans* C18:1 concentration was lower with FAT compared with CON, with lower concentrations of all major *trans* C18:1 isomers (all $P < 0.05$).

The increase in milk fat yield with FAT was because of a 27% increase in the yield of 16-carbon FA compared with CON ($P < 0.001$; Table 5). The FAT treatment had no effect on the yield of de novo FA (<16 carbon FA; $P = 0.32$) or preformed FA (>16 carbon FA; $P = 0.25$) compared with CON. We observed no main effect of FSC on the yield of FA in milk fat derived from de novo (<16 carbon FA), preformed (>16 carbon FA), or mixed (16-carbon FA) sources, but we found a FAT × FSC interaction ($P = 0.03$) such that the yield of 16-car-

bon FA in milk fat was 1.9% lower for HMC compared with DC for CON treatments, whereas 16-carbon FA yield in milk was 4.5% higher for HMC compared with DC for FAT treatments. The FAT treatment increased the yield of C4:0 ($P = 0.04$) but did not affect the yield of de novo FA from C8:0 to *cis*-9 C14:1 (all $P > 0.07$). Yields of C18:0 ($P = 0.18$) and *cis*-9 C18:1 ($P = 0.51$) did not differ for CON and FAT but FAT reduced the yield of total *trans* C18:1 by 9.7% ($P = 0.03$) compared with CON. The FAYR (the additional milk FA yield as a percentage of the additional consumed FA) to FAT was 20.1% for total FA and 29.7% for 16-carbon FA.

DISCUSSION

Understanding the effects of individual FA on production variables of dairy cattle is an area of growing interest. Supplements enriched in SFA may be advanta-

Table 5. Milk FA yield response to C16:0-enriched fat supplement and starch fermentability treatments¹

FA yield (g/d)	CON		FAT		SE	<i>P</i> -value ²		
	DC ²	HMC ³	DC	HMC		FAT	CHO	FAT × CHO
Summations ³								
De novo	284	289	271	262	16.1	0.32	0.75	0.27
Mixed	423	415	536	560	20.8	<0.001	0.29	0.03
Preformed	443	448	429	431	14.0	0.25	0.43	0.64
Selected individual FA								
4:0	31.1	30.2	33.0	32.9	1.27	0.04	0.13	0.31
6:0	21.2	20.8	21.1	20.1	1.06	0.92	0.06	0.43
8:0	12.8	12.7	11.9	11.2	0.72	0.26	0.21	0.29
10:0	33.8	34.1	30.1	28.0	2.32	0.13	0.44	0.26
12:0	40.3	40.7	35.2	33.0	2.76	0.07	0.53	0.32
14:0	136	141	130	128	8.20	0.36	0.53	0.20
14:1 <i>cis</i> -9	9.46	9.17	9.36	8.74	0.52	0.70	0.04	0.52
16:0	408	402	518	542	20.3	<0.001	0.23	0.03
16:1 <i>cis</i> -9	14.4	13.25	18.1	17.75	0.70	<0.001	0.05	0.26
18:0	99.9	108	92.3	94.44	4.67	0.18	0.07	0.23
18:1 <i>trans</i> ⁴	19.1	19.7	17.2	17.7	0.81	0.03	0.12	0.65
18:1 <i>cis</i> -9	219	216	223	225	7.19	0.51	0.87	0.48
18:2 <i>cis</i> -9, <i>cis</i> -12	24.4	23.07	23.5	22.33	0.95	0.41	<0.01	0.92
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	3.13	3.11	2.86	2.75	0.13	0.06	0.35	0.49

¹Treatments were either control (CON; with 2% of diet DM as added soyhulls) or supplemental FA (fatty acid treatment, FAT; with 2% of diet DM as added C16:0-enriched fat supplement). Dry ground corn (DC) grain was fed d 1 to 21 and high-moisture corn (HMC) grain was fed d 22 to 25 of each period.

²FAT = main effect of fat supplementation for DC only (mean of d 18 to 21 of each period); CHO = main effect of fermentable starch challenge (mean of d 22 to 25 of each period); FAT × CHO = interaction of fat supplementation and fermentable starch challenge.

³De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originate from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 + *cis*-9 C16:1).

⁴Total 18:1 *trans* FA.

geous compared with more unsaturated FA supplements because they are relatively rumen inert and do not provide substrate for bioactive products of biohydrogenation. Until recently, most SFA-enriched supplements have contained mixtures of SFA. Our primary objective therefore was to determine the effects of a commercial supplement enriched in C16:0 (~85%) on the yields of milk and milk components. The inclusion of HMC to substitute ground DC in this experiment allowed us to simulate an on-farm situation where fermentability of the ration is altered, increasing the risk of MFD. To our knowledge, no previous studies have evaluated the effects of dietary C16:0 supplementation in combination with diets that may induce MFD.

Milk fat responses to C16:0 supplementation have been variable and therefore not easily predictable. Although some studies have reported an increase in milk fat concentration and yield (Steele and Moore, 1968b; Banks et al., 1976a; Mosley et al., 2007), others have reported little or no response (Storry et al., 1967; Warn-tjes et al., 2008; Rico and Harvatine, 2011) to dietary C16:0 supplementation. In our study, fat concentration and yield increased by 7.2 and 7.3%, respectively, for FAT compared with CON, which was accounted for entirely by the increase in 16-carbon FA in milk. This is in general agreement with results from Mosley et al.

(2007), who reported a greater positive response when feeding a C16:0-enriched supplement at approximately 1.8% of DMI (14.2 and 28.1% increase from control for fat concentration and yield, respectively). The lower magnitude of milk fat response obtained in the present study may be due, at least in part, to the higher starting milk fat yield values of the cows in our study (1.23 vs. 1.01 kg/d) compared with those in Mosley et al. (2007). In addition, compared with non-fat-supplemented control treatments, the C16:0-enriched supplemented diet decreased DMI in the current study, whereas it increased DMI when fed at a similar concentration in Mosley et al. (2007). Further studies are required to determine the basis for variation in milk fat responses across studies.

The effect of fat supplements on DMI is variable and usually depends on the type of fat being fed; however, it mostly results in no change or a decrease in DMI (Allen, 2000). The hypophagic effects of fat feeding have been reported widely and seem to be more pronounced for unsaturated than for saturated FA supplements (Harvatine and Allen, 2006b), with DMI increasing linearly as the degree of unsaturation decreases (Pantoja et al., 1994). Similar studies using C16:0-enriched supplements have reported either no effect (Warn-tjes et al., 2008) or a decrease in DMI (Rico and Harvatine, 2011).

Mosley et al. (2007), however, reported an increase in DMI for cows targeted to consume 500 g/d of a C16:0-enriched supplement and no effect for the higher doses (1,000 and 1,500 g/d) compared with control (no added fat). Our results showed a decrease in DMI for C16:0-supplemented cows (Table 3). It has also been shown that abrupt changes in ruminal fermentability of starch (i.e., replacing DC with HMC) can decrease DMI in dairy cows (Longuski et al., 2009). We observed, however, a FAT $\times$ FSC interaction that suggests that the extent of the response to C16:0 supplementation might change depending on the starch fermentability of the diet. In the present study, the FSC decreased DMI for CON but increased DMI for FAT.

No differences in milk yield were observed with C16:0 supplementation in the current study. This is in agreement with the results of some studies that reported no effect of C16:0 supplementation on milk yield whether it was fed (Steele and Moore 1968a,b; Warntjes et al., 2008) or infused (Enjalbert et al., 1998) to dairy cows. On the contrary, other studies have reported increases in milk yield as a result of C16:0 supplementation (Steele, 1969; Mosley et al., 2007; Piantoni et al., 2013). Mosley et al. (2007) reported an increase in milk yield for a C16:0-enriched fat supplemented at 500 g/d only when fed at 0, 500, 1,000, or 1,500 g/d. The differences in the aforementioned results might indicate differential partitioning of energy coming from the supplemental fat, in some cases mostly incorporated into milk fat and in others partly used as energy for milk synthesis or other purposes. It is possible that circulating C16:0 may have spared some glucose for milk lactose synthesis in the studies that showed a positive milk yield response. The increased glucose availability may be reflected in an increased lactose synthesis and secretion into milk. Mosley et al. (2007) reported an increase in milk yield of 3.1 kg/d when feeding 500 g/d of a C16:0-enriched supplement with a simultaneous increase in milk lactose yield of almost 100 g/d (calculated). Similarly, Steele (1969) reported significant increases in both milk (~ 1 kg/d) and lactose (~ 50 g/d) yields in response to dietary C16:0 supplementation.

Milk protein concentration was slightly lower for C16:0-supplemented cows (Table 3), but milk protein yield was not affected by FAT. Decreases in milk protein concentration have often been reported when fats are supplemented to dairy cows (e.g., Steele, 1969; Wu and Huber, 1994); however, milk protein yield typically remains unchanged (Rabiee et al., 2012). Harvatine and Allen (2006a) reported an increase in milk protein concentration and yield for a saturated versus an unsaturated FA supplement and suggested that the milk protein synthesis response to fat supplementation may

be related to FA saturation. Interestingly, other studies in which C16:0 supplements were fed reported no effect on milk protein concentration and an increase in milk protein yield (Mosley et al., 2007; Warntjes et al., 2008).

Feed efficiency of cows in the present study was significantly higher when the C16:0-enriched supplement was fed, as a result of the lower DMI and an increase in 3.5% FCM (Table 3). Similarly, we also reported an increase in feed efficiency when feeding a pure C16:0 fat supplement compared with soyhulls, but in this case there was no reduction in DMI and the increase in efficiency was a result of the increase in the yield of milk and milk fat (Piantoni et al., 2013). In this latter study, dry ground corn was the major starch source, similar to the current study during d 1 to 21 of each period, when an improvement in feed efficiency was observed. Interestingly, we observed a FAT $\times$ FSC interaction in the current study, indicating that the feed efficiency response was affected by the fermentability of the corn in the diet, a finding that requires further investigation.

A review by Grummer (1991) showed that fat supplementation usually decreases the proportion of de novo FA (C6:0 to C16:0) in milk fat in a linear fashion with increasing fat supplementation. These effects have been reported when feeding both saturated and unsaturated FA supplements, the latter having the most dramatic effects on de novo FA synthesis (Banks et al., 1976b; Clapperton and Banks, 1985). Our results are in agreement with the aforementioned studies (Table 4); the concentrations of all FA from C6:0 to *cis*-9 C14:1 decreased as a result of feeding the C16:0-enriched supplement. The concentration of C4:0, however, was not different for FAT and CON treatments, following a different trend from all other de novo FA in milk fat. Moreover, the yield (g/d) of C4:0 was higher in FAT compared with CON (Table 5), which is in agreement with previous results (Enjalbert et al., 1998; Mosley et al., 2007). It has been suggested that the increased yield of C4:0 might be part of a mechanism to maintain milk fat fluidity at body temperature, with an increase in C4:0 output due to the large diglyceride pool of high-molecular-weight FA that results from the incorporation of long-chain FA taken up from plasma (Barbano and Sherbon, 1980). The results from Enjalbert et al. (1998) seem to be in agreement with this proposed mechanism because they reported that correlations between the mammary uptake (mmol/L milk) of C16:0 and C18:0 (high-melting-point FA) and C4:0 balance (mmol/L milk) were higher than correlations between *cis*-9 C18:1 uptake and C4:0 balance. In addition, in vitro studies have reported a strong stimulatory effect of C16:0 on C4:0 synthesis and incorporation into triglyc-

erides in dispersed bovine mammary gland cells, and to a smaller extent, increased synthesis and incorporation of C16:0 itself into triglycerides (Hansen and Knudsen, 1987a,b). It is likely that C16:0 has the same effects *in vivo*, which is evidenced by the increased yield (g/d) of both C4:0 and C16:0 observed with FAT in our study (Table 5) and Mosley et al. (2007) in response to C16:0 supplementation.

The decrease in the concentration of other *de novo* FA (C6:0 to C14:0) in milk fat observed in the present study (Table 4) has been reported by other authors (Mosley et al., 2007; Warntjes et al., 2008); however, it does not always occur with a simultaneous decrease in their yield (Enjalbert et al., 2000). It has been proposed that C16:0 supplementation may decrease the activity of acetyl-CoA carboxylase or fatty acid synthase due to allosteric inhibition by C16:0 (Wright et al., 2002), or inhibit mammary gland acetyl-CoA carboxylase by palmitoyl-CoA derived from C16:0 (Miller et al., 1970). However, in the current study, C16:0 supplementation did not decrease the yield of C6:0 to *cis*-9 C14:1 although we observed a slight nonsignificant reduction in overall *de novo* FA yield of 4.9% (Table 5).

We used FAYR to the C16:0-enriched supplement to evaluate the efficiency of utilization of supplemental FA for milk FA synthesis. The FAYR for total FA and 16-carbon FA was 20.1 and 29.7%, respectively, when cows received FAT. When estimated from results reported by Mosley et al. (2007), FAYR to a C16:0-enriched supplement fed at 1.0 and 1.5 kg/d were 40 and 33% for total FA and 38 and 30% for 16-carbon FA, respectively. When approximately 500 g of C16:0 was infused in the duodenum, the FAYR for 16-carbon FA was 47% (Enjalbert et al., 2000). Interestingly, Piantoni et al. (2013) observed lower FAYR of 11.7 and 16.5% for total FA and 16-carbon FA, respectively, when a 99% pure C16:0 supplement was fed to cows with a wide range of milk production. In contrast, a FAYR for total FA of 30% was observed when an SFA supplement containing a mixture of C16:0 and C18:0 was fed (Relling and Reynolds, 2007). It remains to be determined whether FAYR to dietary C16:0 and C18:0 differ. Further studies aimed at improving our understanding of the wide variation in these responses and their consistency are vital for the efficient and economical use of C16:0-enriched supplements on commercial dairy farms.

Our secondary objective was to utilize an FSC to induce MFD, similar to previous experiments that used a highly fermentable starch sources such as HMC (Longuski et al., 2009). Milk fat depression, however, was not observed in the present study, which did not permit the assessment of the effects of C16:0 feeding

in an MFD situation. Similarly, Krause et al. (2002a) reported no effect of replacing DC by HMC on milk fat concentration and yield, despite a reduction in rumen pH in the HMC treatment (Krause et al., 2002b). Reasons for the lack of MFD response to a change in carbohydrate fermentability in our study are not clear; DM concentrations for both DC and HMC were within common ranges, at 87% and 67% DM, respectively. It is likely that the fiber concentration of the ration (30.8% NDF and 20.4% forage NDF for the HMC diet in the FAT treatments) was adequate to maintain rumen pH and this, combined with low dietary unsaturated FA available for rumen metabolism, might have prevented MFD when cows were challenged with HMC. Further studies with lower NDF diets, the use of highly fermentable starch sources, and a higher content of unsaturated FA might successfully induce MFD to determine the effects of a C16:0-enriched supplement under such circumstances. Although the potential exists for a C16:0-enriched supplement to increase milk fat yield, we are not suggesting that fat supplementation be used to increase milk fat yield under MFD situations. Rather, the cause of the MFD should be identified and rations reformulated to prevent MFD.

CONCLUSIONS

Results from this experiment demonstrate the potential for a dietary C16:0-enriched fat supplement to improve milk fat concentration and yield, as well as efficiency of feed conversion into milk. Further studies are required to verify and extend these results and to determine whether responses are similar across different diets, levels of milk production, and stages of lactation. Effects of dietary C16:0-enriched fat supplements compared with supplements containing other long-chain FA, such as C18:0, are also required.

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