Compared with stearic acid, palmitic acid increased the yield of milk fat and improved feed efficiency across production level of cows

J. E. Rico, M. S. Allen, and A. L. Lock¹

Department of Animal Science, Michigan State University, East Lansing 48824

ABSTRACT

The effects of dietary palmitic and stearic acids on feed intake, yields of milk and milk components, and feed efficiency of dairy cows were evaluated in an experiment with a crossover arrangement of treatments with a covariate period. Cows with a wide range of milk production (38 to 65 kg/d) were used to determine if response to fat supplementation varied according to production level. Thirty-two Holstein cows (143 ± 61 d in milk) were assigned randomly to a treatment sequence within level of milk production. Treatments were diets supplemented (2% of diet dry matter) with palmitic acid (PA; 97.9% C16:0) or stearic acid (SA; 97.4% C18:0). Treatment periods were 21 d and cows were fed a nonfat supplemented diet for 14 d immediately before the first treatment period. The final 4 d of each period were used for sample and data collection. Milk production measured during the covariate period (preliminary milk yield) was used as the covariate. No interactions were detected between treatment and preliminary milk yield for the production response variables measured. Compared with SA, the PA treatment increased milk fat concentration (3.66 vs. 3.55%) and yield (1.68 vs. 1.59 kg/d), and 3.5% fat-corrected milk yield (47.5 vs. 45.6 kg/d). Treatment did not affect dry matter intake, milk yield, milk protein yield, body weight, or body condition score. Milk protein concentration was lower for PA compared with SA treatment (3.24 vs. 3.29%). The PA treatment increased feed efficiency (3.5% fat-corrected milk yield/dry matter intake) compared with SA (1.48 vs. 1.40). The increase in milk fat yield by PA was entirely accounted for by a 24% increase in 16-carbon fatty acid output into milk. Yields of de novo (3.2%) and preformed fatty acids (2.9%) were only slightly decreased by PA relative to SA. The PA treatment increased plasma concentration of nonesterified fatty acids (96.3 vs. 88.2 $\mu\text{Eq/L}$) and glucose (56.6 vs. 55.7 mg/dL) compared with SA, but insulin and β -hydroxybutyrate were not altered by the

treatments. Results demonstrate that palmitic acid is more effective than stearic acid in improving milk fat concentration and yield as well as efficiency of feed conversion to milk. Responses were independent of production level and without changes in body condition score or body weight. Further studies are required to test the consistency of these responses across different types of diets.

Key words: milk fat, palmitic acid, stearic acid, production level

INTRODUCTION

The effect of supplementing fats in lactating dairy cow rations has been widely studied. Fat supplements are often used because they increase the dietary energy density of the diet and can improve energy balance, feed efficiency, and the yields of milk and milk fat (Rabiee et al., 2012). However, the effects of individual FA have not yet been adequately studied to successfully allow for the identification of specific FA that maximize the yield of milk and milk components. The concentration and yield of milk fat can be increased by feeding SFA supplements to dairy cows, compared with both control (non-added-fat diets) and unsaturated FA supplements (Relling and Reynolds, 2007). Even though the effects of SFA supplements have been reported in numerous studies, it is still unclear what effects individual SFA have, with only a limited number of studies available that used pure sources of individual SFA, such as palmitic (C16:0) and stearic (C18:0) acids (e.g., Steele and Moore, 1968; Enjalbert et al., 1998).

Recent research has focused on studying the effects of C16:0, using C16:0-enriched fat supplements. This FA has been reported to increase milk yield, milk fat concentration and yield, and the efficiency of milk production (Mosley et al., 2007; Lock et al., 2013; Piantoni et al., 2013). Information on the effects of feeding C18:0, particularly when compared with C16:0, is much more limited, with only a few studies available. A series of studies in the 1960s, feeding relatively pure sources of SFA to low-producing dairy cows, reported that both C16:0 and C18:0 increased milk fat concentration and yield relative to control. The responses to C16:0 feed-

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¹Corresponding author: allock@msu.edu

ing, however, were higher and more consistent across studies, compared with C18:0 (Steele and Moore, 1968; Steele, 1969). These results are supported by Enjalbert et al. (1998), who reported higher milk fat concentration and yield using duodenal infusions of C16:0 and C18:0 in cows compared with controls. Moreover, C16:0 had higher uptake efficiency by the mammary gland compared with C18:0 in that study. In recent studies, the increased milk fat concentration and yield in response to C16:0 supplementation was explained entirely by the increase in 16-carbon FA secreted into milk (Lock et al., 2013; Piantoni et al., 2013). The aforementioned results suggest that C16:0 feeding will increase milk fat content and yield compared with C18:0 because of a greater incorporation of C16:0 into milk fat.

A limited number of studies have evaluated the effect of milk production of cows on their response to fat feeding. Harvatine and Allen (2005) reported a greater increase in milk protein yield in high-producing cows relative to low-producing cows in response to a saturated fat supplement compared with an unsaturated fat supplement. In a field study, Warntjes et al. (2008) reported that cows with a lower milk yield had a higher milk production increase in response to C16:0-enriched fat supplementation relative to cows with a higher milk production. However, Piantoni et al. (2013) reported that production responses to a pure C16:0 fat supplement compared with soyhulls did not differ across production levels of cows.

To our knowledge, no previous studies have directly compared the effects of C16:0 and C18:0 across a wide range of milk production levels in dairy cows. Therefore, our objective was to compare the effects of C16:0 and C18:0 supplementation on feed intake and production responses in dairy cows with a wide range of milk production levels. We hypothesized that C16:0 feeding would increase milk fat content and yield compared with C18:0 due to a greater incorporation of C16:0 into milk fat, and that responses would be similar across production levels.

MATERIALS AND METHODS

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). Thirty-two multi- and primiparous Holstein cows averaging 143 ± 61 DIM (mean $\pm$ SD) with milk production ranging from 38 to 65 kg/d from the Michigan State University Dairy Field Laboratory were randomly assigned to treatments within level of milk production. At the beginning of the experiment, the BW of cows was 713 ± 76 kg, milk

yield was 50.2 ± 8.8 kg/d, milk fat concentration was $3.13\% \pm 0.58\%$, and milk fat yield was 1.54 ± 0.28 kg/d (mean $\pm$ SD).

The experiment used a crossover arrangement of treatments in 2 consecutive 21-d periods, preceded by a 14-d covariate period. Animals received a common diet with no fat supplementation during the covariate period to obtain baseline values, and were then randomly allocated to either a C16:0-enriched fat-supplemented [palmitic acid (PA) treatment] or C18:0-enriched fat-supplemented [stearic acid (SA) treatment] diet for period 1 and switched to the other diet during period 2. The C16:0 supplement (Emery Oleochemicals, Selangor, Malaysia) and C18:0 supplement (Acidchem International Sdn Bhd, Penang, Malaysia) were both free fatty acid products of high purity and contained more than 97% of either C16:0 or C18:0, respectively, and 99% total SFA.

Diets were based on corn silage as the major forage component and dry ground corn grain as the major concentrate component of the diet (Table 1). The C16:0-enriched fat supplement and the C18:0-enriched fat supplement were included in the diet at 2% of DM (Table 1). Diets were formulated to contain 30% NDF and 16% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. Dry matter concentration was determined twice weekly for forages, and diets were adjusted when necessary.

Data and Sample Collection

Cows were housed in individual tiestalls throughout the experiment. Access to feed was blocked from 0800 to 1000 h to allow for orts and feed offered to be weighed daily. Cows were fed 115% of expected intake at 1000 h daily and feed intake was recorded and adjusted daily. Cows were milked twice daily at 0430 and 1530 h, with milk yield recorded electronically (Boumatic ProVantage Dairy Management System, Boumatic, Madison, WI) at each milking throughout the experiment. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice per day.

Response variables were averaged for d 11 to 14 of the covariate period and for d 18 to 21 of each of the treatment periods. Diet ingredients and orts were sampled during d 18 to 21 and composited for each period. Milk was sampled at each milking from d 11 to 14 of the covariate period and from d 18 to 21 of the treatment periods. 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

Table 1. Ingredients and nutrient composition (% of DM unless otherwise noted) of experimental diets supplemented with palmitic (PA; C16:0) and stearic acid (SA; C18:0)

Item	Diet	
	PA	SA
Ingredient		
Corn silage	26.1	26.1
Alfalfa silage	7.4	7.4
Chopped alfalfa hay	6.6	6.6
Dry ground corn	25.9	25.9
Soybean meal	12.8	12.8
Cottonseed with lint	3.6	3.6
Vitamin and mineral mix ¹	8.6	8.6
Soyhulls	7.0	7.0
Fat supplement ²	2.0	2.0
Nutrient composition		
DM (%)	58.3	58.3
NDF	29.7	29.7
Forage NDF	19.1	19.1
Forage NDF (% of NDF)	64.2	64.2
CP	15.0	15.0
Starch	27.1	27.1
Total FA	4.42	4.42
16:0	2.35	0.45
18:0	0.12	2.01

¹Vitamin 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 trace minerals, biotin, vitamin E, vitamin A, vitamin D, and selenium.

²Palmitic acid supplement contained 97.9% C16:0; SA supplement contained 97.4% C18:0.

FA composition. Blood samples were taken every 15 h during the last 4 d of each period to obtain a 24-h composite sample, representing intervals of 3 h between samples. Blood was collected by coccygeal venipuncture into 3 evacuated tubes; 2 contained potassium EDTA as an anticoagulant and the other contained potassium oxalate as an anticoagulant and sodium fluoride as a glycolytic inhibitor. Blood was stored on ice until centrifugation at $2,000 \times g$ for 15 min at 4°C (within 30 min of sample collection). Body weights were measured on the last 2 d of the covariate period and on d 20 and 21 of each of the treatment periods. Three trained investigators determined BCS on a 5-point scale (in 0.25-point increments; Wildman et al., 1982) on the last day of each period.

Sample Analysis

Diet ingredients and orts 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 Harvatiné and Allen (2005). Fatty acid concentrations of feed ingredients

were determined using a modification of the one-step transesterification method developed by Sukhija and Palmquist (1988) as described previously by Lock et al. (2013).

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. Lipids were extracted and methylated, and composition of FA methyl esters was determined by GLC, according to the methods described by Lock et al. (2013). Short-chain FA methyl esters 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).

Plasma samples were analyzed in duplicate using commercial kits to determine the plasma concentrations of NEFA (NEFA-HR kit, Wako Chemicals USA, Richmond, VA; intraassay CV = 2.7%), BHBA (procedure #2440, Stanbio Laboratory, Boerne, TX; intraassay CV = 3.1%), and insulin (Coat-A-Count RIA kit, Siemens Healthcare Diagnostics, Deerfield, IL; intraassay CV = 9.0%). Plasma glucose concentration was analyzed using a glucose oxidase method (PGO Enzyme, P7119, Sigma Chemical Co., St. Louis, MO; intraassay CV = 1.1%).

Statistical Analysis

All data were analyzed using the fit model procedure of JMP (version 9.0.2, SAS Institute Inc., Cary, NC) according to

$$Y_{ijk} = \mu + C_i + P_j + T_k + P_j \times T_k + \text{pMY} + \text{pMY} \times T_k + \text{pMY}^2 + \text{pMY}^2 \times T_k + e_{ijk},$$

where Y_{ijk} = the dependent variable; μ = the overall mean; C_i = the random effect of cow ($i = 1-32$); P_j = the fixed effect of period ($j = 1-2$); T_k = the fixed effect of treatment ($k = 1-2$); pMY = preliminary milk yield used as covariate; $\text{pMY} \times T_k$ = the interaction

Table 2. Fatty acid concentration and intake of palmitic (PA) and stearic acid (SA) diets fed during the treatment periods

Item	Treatment ¹	
	PA	SA
FA concentration (g/100 g of total FA)		
16:0	53.2	10.2
18:0	2.6	45.6
18:1 <i>cis</i> -9	12.2	12.2
18:2 <i>cis</i> -9, <i>cis</i> -12	26.2	26.2
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	2.6	2.6
Σ Other	3.1	3.3
Σ SFA	57.0	57.1
Σ MUFA	13.9	13.9
Σ PUFA	29.1	29.0
FA intake (g/d) ²		
Total	1,412	1,421
16:0	752	144
18:0	37	648
18:1 <i>cis</i> -9	173	173
18:2 <i>cis</i> -9, <i>cis</i> -12	370	372
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	37	37
Σ Other	44	47
Σ SFA	805	811
Σ MUFA <i>cis</i>	197	197
Σ PUFA <i>cis</i>	411	413

¹Treatments were either PA (with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or SA (with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

²Fatty acid intake expressed as grams per day of individual FA; calculated based on DMI for each treatment.

between preliminary milk yield and treatment ; pMY^2 = preliminary milk yield squared; $pMY^2 \times T_k$ = the interaction between preliminary milk yield squared and treatment ; and e_{ijk} = the residual error. Normality of the residuals was checked with normal probability and

box plots and homogeneity of variances with plots of residuals versus predicted values. Main effects were declared significant at $P \leq 0.05$ and trends were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$ and trends were declared at $P \leq 0.15$. Interactions were evaluated but removed from the statistical model when not significant ($P > 0.15$). In general, period by treatment interaction was not significant, but variables with significant interactions are noted in the tables. All data was expressed as LSM and SEM, unless otherwise specified.

RESULTS

Production Responses

Nutrient composition of experimental diets supplemented with C16:0 and C18:0 are reported in Table 1. The concentrations of C16:0 and C18:0 were 2.35 and 2.01% of ration DM for PA and SA, respectively. Relative to SA, the PA treatment increased total daily C16:0 intake ~5 fold. The SA treatment increased total daily C18:0 intake ~17 fold. Total FA intake and intake of other FA was similar across diets (Table 2).

We observed no interaction between preliminary milk yield (**pMY**) and treatment for any of the production responses evaluated (Table 3). Thus, the remainder of the production results will concentrate on main effects of the PA and SA treatments.

Compared with SA, PA increased milk fat concentration by 3.1%, from 3.55 to 3.66% ($P = 0.01$), and milk fat yield by 5.7%, from 1.59 to 1.68 kg/d ($P < 0.0001$;

Table 3. Dry matter intake, milk production and composition, feed efficiency, BW, and BCS for cows fed treatment diets (n = 32)

Item	Treatment ¹		SEM	Treatment <i>P</i> -value
	PA	SA		
DMI (kg/d)	32.1	32.3	0.44	0.39
Milk yield (kg/d)				
Milk	46.6	45.8	2.02	0.22
3.5% FCM	47.5	45.6	1.64	<0.01
ECM	47.7	46.1	1.63	<0.01
Feed efficiency ²	1.48	1.40	0.05	<0.0001
Milk component composition (%)				
Fat	3.66	3.55	0.09	0.01
Protein	3.24	3.29	0.05	<0.01
Lactose	4.75	4.76	0.04	0.71
Milk component yield (kg/d)				
Fat	1.68	1.59	0.05	<0.0001
Protein	1.50	1.49	0.05	0.70
Lactose	2.22	2.18	0.10	0.24
BW (kg)	720	723	13.6	0.12
BCS	2.93	2.99	0.11	0.11

¹Treatments were either palmitic acid (PA; with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or stearic acid (SA; with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

²Feed efficiency = kg of 3.5% FCM/kg of DMI.

Table 3). Consequently, PA increased 3.5% FCM yield relative to SA by 1.9 kg/d (4.2%; $P < 0.01$) and ECM by 1.6 kg/d (3.5%; $P < 0.01$). No differences were observed between the treatments for yields of milk, milk protein, or milk lactose (all $P > 0.20$). The PA treatment decreased milk protein concentration relative to SA ($P < 0.01$) from 3.29 to 3.24%. Dry matter intake was not different between treatments ($P = 0.39$); however, the increase in 3.5% FCM with the PA treatment resulted in a 5.7% improvement in feed efficiency (3.5% FCM/DMI) compared with SA ($P < 0.001$; Table 3). Body weight and BCS were not affected by treatment (both $P > 0.11$).

Plasma Metabolites and Hormones

Relative to SA, PA increased the concentration of plasma glucose by 1.5% ($P < 0.05$). Similarly, compared with SA, PA increased NEFA concentration (9.2%; $P < 0.01$), and this response was affected by pMY with a greater effect as milk yield increased (pMY $\times$ treatment interaction; $P < 0.05$). We observed no effects of treatment on plasma insulin or BHBA ($P > 0.80$; Table 4).

Milk FA Concentrations and Yields

Concentrations and yields of selected FA are shown in Tables 5 and 6, respectively. Relative to SA, PA increased the concentration of C16:0 and *cis*-9 C16:1 in milk fat by 18 and 12%, respectively (both $P < 0.0001$), and decreased the concentrations of most other milk FA (Table 5). On the contrary, relative to PA, SA increased the concentration of C18:0 and *cis*-9 C18:1 in milk fat by 18 and 7%, respectively (both $P < 0.0001$; Table 5).

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 the treatments. For PA and SA, the FA profile (g/100 g of total FA) of milk

fat was 28.0 and 30.5 de novo FA (<16 carbon; $P < 0.0001$), 38.6 and 32.8 16-carbon FA ($P < 0.0001$), and 34.8 and 35.3 preformed FA (>16 carbon; $P < 0.001$), respectively (Table 5).

The increase in milk fat yield with PA (90 g/d) corresponded to a 24% increase in the yield of 16-carbon FA relative to SA (117 g/d increase, $P < 0.0001$; Table 6). We observed a simultaneous decrease in the yields of de novo FA (<16 carbon; 3.2%, $P < 0.05$) and preformed FA (>16 carbon; 2.9%, $P < 0.05$) relative to SA. The yields of de novo FA in milk fat from C8:0 to C14:0 were all decreased by PA relative to SA (all $P < 0.05$), whereas C6:0 was unchanged ($P = 0.54$). We observed an increase in the yield of C4:0 for PA relative to SA (6.2%; $P < 0.001$), which was different from all other de novo FA in milk fat. As the chain length of de novo FA increased, their reduction in yield in PA compared with SA increased linearly (Figure 1). The SA treatment increased the yield of C18:0 by 11.2% relative to PA ($P < 0.0001$). However, this increase of 13 g/d of C18:0 in the SA treatment was much less than the 113 g/d increase in C16:0 with the PA treatment. We observed no effect of treatments on the yield of *cis*-9 C18:1 ($P = 0.47$) or total *trans* C18:1 ($P = 0.75$).

DISCUSSION

The use of purified sources of C16:0 and C18:0 in the present experiment allowed us to directly compare the effects of these FA while avoiding the potential confounding effects of other FA in the fat supplements. Therefore, the diets differed only in the inclusion of either C16:0 or C18:0. Previous research has shown that the production and metabolic responses to fat supplements can differ in cows at different levels of milk production (Harvatine and Allen, 2005; Warntjes et al., 2008). Preliminary milk yield was chosen as a covariate in the present experiment to determine any interactions between treatment and cow production level. Additionally, this information is readily available to the dairy producer and can be easily used for grouping and feeding cows. Cows in the present experiment responded

Table 4. Plasma metabolites and hormones of cows fed treatment diets (n = 32)

Item	Treatment ¹		SEM	Treatment <i>P</i> -value
	PA	SA		
Glucose (mg/dL)	56.6	55.7	0.84	0.04
NEFA ² (μ Eq/L)	96.3	88.2	4.10	0.008
Insulin (μ IU/mL)	9.60	9.10	0.89	0.90
BHBA (mg/dL)	5.20	5.00	0.38	0.88

¹Treatments were either palmitic acid (PA; with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or stearic acid (SA; with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

²Preliminary milk yield (pMY): $P = 0.77$; pMY $\times$ treatment: $P = 0.03$.

Table 5. Milk FA concentrations of cows fed treatment diets (n = 32)

FA concentration (g/100 g)	Treatment ¹		SEM	Treatment <i>P</i> -value
	PA	SA		
Selected individual FA ²				
4:0	3.03	3.02	0.06	0.81
6:0	2.11	2.21	0.04	<0.0001
8:0	1.27	1.39	0.03	<0.0001
10:0	3.25	3.67	0.10	<0.0001
12:0	3.85	4.38	0.13	<0.0001
14:0	11.1	12.1	0.16	<0.0001
14:1 <i>cis</i> -9	0.94	0.97	0.05	0.03
16:0	36.7	31.2	0.41	<0.0001
16:1 <i>cis</i> -9	1.81	1.62	0.06	<0.0001
18:0 ³	7.39	8.72	0.24	<0.0001
18:1 <i>trans</i> ⁴	1.86	1.97	0.10	<0.01
18:1 <i>cis</i> -9 ⁵	16.7	17.9	0.27	<0.0001
18:1 <i>cis</i> -11	0.57	0.60	0.02	<0.01
18:2 <i>cis</i> -9, <i>cis</i> -12	2.63	2.87	0.05	<0.0001
18:2 <i>cis</i> -9, <i>trans</i> -11	0.33	0.34	0.02	0.32
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.32	0.36	0.01	<0.0001
Summations ⁶				
De novo	28.0	30.5	0.40	<0.0001
Mixed	38.6	32.8	0.43	<0.0001
Preformed	34.8	35.3	0.43	<0.0001
Σ SFA	71.1	69.4	0.45	<0.0001
Σ MUFA <i>cis</i>	21.3	22.4	0.35	<0.0001
Σ PUFA <i>cis</i>	3.44	3.75	0.06	<0.0001
Σ Unknown	0.62	0.64	0.02	0.01
Σ Others ⁷	6.15	6.62	0.09	<0.0001

¹Treatments were either palmitic acid (PA; with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or stearic acid (SA; with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

²A total of approximately 70 individual FA were quantified and used for calculations (summation by source and concentrations). Only select FA are reported in the table.

³Preliminary milk yield (pMY): $P = 0.21$; pMY $\times$ treatment interaction: $P = 0.02$.

⁴Total 18:1 *trans* FA.

⁵pMY: $P = 0.04$; pMY $\times$ treatment interaction: $P = 0.08$.

⁶De 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).

⁷Consists of 44 identified FA ranging from C5:0 to C22:5.

similarly to treatment (PA or SA), as evidenced by a lack of interaction between treatment and pMY for all production variables and most other variables evaluated, with the exception of plasma NEFA and the concentration and yield of some FA in milk. These results are similar to Piantoni et al. (2013), who reported no interaction between pMY and production responses to C16:0 feeding compared with a soyhulls control diet.

Fat supplements, including mostly SFA, can increase both milk fat concentration and yield (Christensen et al., 1994; Relling and Reynolds, 2007). With the high-producing cows in our experiment, fat concentration and yield increased by 3.1 and 5.7% for PA relative to SA, respectively, which was completely accounted for by an increase in 16-carbon FA secretion into milk. We have reported similar results when C16:0-enriched supplements have been fed to dairy cows at 2% of ration DM, compared with non-added-fat controls (Lock et al., 2013; Piantoni et al., 2013). Importantly, the

extent of the milk fat response in our experiment was very similar to that reported in those studies (100 and 80 g/d for Lock et al., 2013 and Piantoni et al., 2013, respectively), even though the treatment comparison used in our experiment was isocaloric (PA vs. SA). Steele and Moore (1968) and Steele (1969) evaluated the effects of feeding C16:0 or C18:0 at approximately 4% of ration DM to low-producing dairy cows. Relative to control, C16:0 increased milk fat concentration and yield consistently across the 2 studies. However, responses to C18:0 were inconsistent because C18:0 did not increase milk fat concentration and it increased fat yield in only 1 of the 2 studies. Moreover, C18:0 increased milk fat yield to a lesser extent than C16:0.

Fat supplements can sometimes, but not always, reduce DMI, and the response usually depends on the type of fat being fed (Allen, 2000; Relling and Reynolds, 2007). The hypophagic effects of FA seem to be more pronounced for unsaturated FA than for SFA supple-

Figure 1. Change in milk FA yield in relation to FA carbon chain length [calculated as the difference in the yield of each FA, palmitic acid (PA) minus stearic acid (SA) treatment]. Treatments were either PA (with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or SA (with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

ments (Drackley et al., 1992). Previous studies that compared C16:0-enriched supplements with non-added-fat controls have reported either no effect (Warntjes et al., 2008; Piantoni et al., 2013) or a decrease in DMI (Rico and Harvatine, 2011; Lock et al., 2013). The lack of difference between PA and SA on DMI in our experiment suggests that the effects of C16:0 and C18:0 on DMI are similar. Feed efficiency, however, was affected by treatment, which is explained by an increase in 3.5% FCM yield for PA, relative to SA, with similar DMI across treatments (Table 3). This is similar to Piantoni et al. (2013), who also reported increased feed efficiency as a result of an increase in 3.5% FCM and no change in DMI when C16:0 was supplemented at 2% of ration DM.

The observed lack of effects of treatment on milk yield is in line with the results from Steele (1969), who reported that C16:0 and C18:0 increased milk yield in a manner similar to a control diet that did not include added fat. When individually compared with non-added-fat control diets, both C16:0- and C18:0-enriched supplements have increased (Steele, 1969; Mosley et al., 2007; Piantoni et al., 2013) or had no effect on milk yield (Steele and Moore 1968; Warntjes et al., 2008; Lock et al., 2013). These differences may suggest differential partitioning of the energy supplied through the supplemental FA, sometimes more efficiently directed toward milk fat and in some cases allowing for more energy to be available for milk synthesis or other uses

in different tissues. For example, it has been suggested that the suppression of de novo synthesis of FA in the mammary gland that may occur when long-chain FA are fed can decrease the oxidative use of glucose to generate reducing equivalents for milk fat synthesis (Storry et al., 1973). This could occur through direct inhibition of acetyl CoA carboxylase by long-chain FA inhibition, resulting in a reduced use of glucose through the pentose phosphate pathway to yield NADPH (Palmquist and Jenkins, 1980). Therefore, in those experiments that reported positive milk yield responses relative to controls, C16:0 and C18:0 may have partially spared glucose, allowing its utilization in other milk synthetic processes.

Lowered plasma insulin concentrations in lactating cows fed fat supplements have been reported previously (Choi and Palmquist, 1996; Choi et al., 2000) when a calcium soap of long-chain FA was included in the diet to replace starch. Carbohydrate supply in our experiment was the same across treatments, which may explain the lack of difference in insulin concentration between PA and SA. Similarly, this may also indicate that C16:0 and C18:0 have similar effects on insulin secretion. Cant et al. (1993) concluded that an increased mammary supply of FA from fat supplementation spared acetate for oxidation by extramammary and extrahepatic tissues, thus increasing the efficiency of glucose use for lactose synthesis. In our experiment, the slight increase in plasma glucose in PA, relative to SA, was not sufficient to drive increased concentrations of either plasma insulin or milk lactose yield, consistent with the lack of difference in milk yield.

Plasma NEFA have been reported previously to increase as a result of adding saturated fat to diets (Drackley et al., 1992; Choi and Palmquist, 1996; Choi et al., 2000) and specifically for C16:0-supplemented cows (Piantoni et al., 2013). Additionally, evidence exists that mammary lipid secretion depends moderately on the availability of preformed FA, such as plasma circulating NEFA, which in turn are regulated by insulin (Rigout et al., 2002; Bauman and Grinari, 2003; Corl et al., 2006). The increase (9.2%) in plasma NEFA observed in the present experiment may have contributed to a greater supply of preformed FA, particularly of C16:0, to the mammary gland, explaining part of the observed increase in milk fat secretion with the PA treatment compared with SA. Moreover, the lack of differences in BW and BCS between treatments suggests that the changes in plasma NEFA are not due to effects of treatment on the mobilization of body fat reserves.

Extensive work has been done in evaluating the effects of dietary fat on milk fat composition and yield (see reviews by Grummer, 1991 and Palmquist et al., 1993). It is widely recognized that lipid supplementa-

Table 6. Milk FA yields of cows fed treatment diets (n = 32)

FA yield (g/d)	Treatment ¹		SEM	Treatment P-value
	PA	SA		
Summations ^{2,3}				
De novo	441	455	17.0	0.04
Mixed	608	491	21.0	<0.0001
Preformed	524	539	16.0	0.04
Selected individual FA				
4:0	47.9	45.1	1.94	<0.001
6:0	33.4	33.1	1.40	0.54
8:0	20.1	20.8	0.88	0.03
10:0	51.2	54.8	2.51	<0.001
12:0	60.4	65.3	2.92	<0.0001
14:0	175	181	6.92	0.02
14:1 <i>cis</i> -9	14.6	14.3	0.16	0.40
16:0	580	467	20.2	<0.0001
16:1 <i>cis</i> -9	28.3	23.8	1.08	<0.0001
18:0	116	129	5.20	<0.0001
18:1 <i>trans</i> ⁴	29.3	29.0	0.39	0.75
18:1 <i>cis</i> -9	260	262	7.48	0.47
18:2 <i>cis</i> -9, <i>cis</i> -12	41.4	42.6	1.61	0.09
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	5.07	5.35	0.21	0.002

¹Treatments were either palmitic acid (PA; with 2% of diet DM as added palmitic acid supplement; 97.9% C16:0) or stearic acid (SA; with 2% of diet DM as added stearic acid supplement; 97.4% C18:0).

²A total of approximately 70 individual FA were quantified and used for calculations (summation by source and concentrations). Only select FA are reported in the table.

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.

tion can decrease de novo FA concentration and yield (Glasser et al., 2008). Our results are in agreement with the aforementioned reviews as the concentration and yield of de novo FA decreased with PA, relative to the SA treatment. However, the yield of C4:0 was higher in PA relative to SA (Figure 1, Table 6), suggesting that incorporation of this FA was favored by additional C16:0 entering the mammary gland, in contrast to the effects observed for other de novo FA. The increase in C4:0 yield in our experiment is in agreement with previous reports from studies that supplemented C16:0-enriched fats (Mosley et al., 2007; Lock et al., 2013; Piantoni et al., 2013). Barbano and Sherbon (1980) suggested that increased secretion of C4:0 into milk fat might be part of a mechanism to help maintain milk fat fluidity at body temperature, as long-chain FA of high melting point are taken up from plasma when additional long-chain FA are fed. In addition, it has been reported that C16:0 can have a strong stimulatory effect on the synthesis of C4:0, as well as on its incorporation into triglycerides in dispersed bovine mammary gland cells, and to a smaller extent, an increased synthesis and incorporation of C16:0 itself into triglycerides (Hansen and Knudsen, 1987a,b). The increased yield of C4:0 and C16:0 in our experiment is in agreement with the proposed stimulatory effects of C16:0 on incorporation of these FA into milk triglycerides.

The observed decrease in the yield of other de novo synthesized FA, however, could be explained by several findings. Lock et al. (2013) discussed possible mechanisms implicated in the reduction of de novo FA synthesis, which may include an inhibitory effect of C16:0 supplementation on activity of acetyl-CoA carboxylase or FA synthase due to allosteric inhibition by C16:0 (Wright et al., 2002) or the inhibition of mammary gland acetyl-CoA carboxylase by palmitoyl-CoA derived from C16:0 (Miller et al., 1970). In this context, the observed increase in C4:0 yield in milk fat with PA observed in our experiment might indicate reduced activity of either acetyl-CoA carboxylase or FA synthase, and thus lead to reduced de novo FA synthesis. In addition, Hansen and Knudsen (1987a) proposed that the most likely explanation for the inhibitory effect of certain FA on the rate of de novo synthesis is that they compete with newly synthesized medium-chain acyl-CoA for the *sn*-2 and *sn*-3 positions of the triglyceride backbone during the esterification steps of mammary lipid synthesis. Breckenridge and Kuksis (1968) reported that approximately 90% of the FA from C4:0 to C10:0 were incorporated into the *sn*-3 position and that placement of C4:0 into this position in particular is highly selective. Additionally, the placement of some 18-carbon FA and C14:0 is also selective, with C18:0 and C18:1 occurring preferentially in *sn*-1 position, 14:0 in *sn*-2, and C16:0

equally distributed in *sn*-1 and *sn*-2 but poorly incorporated into *sn*-3 (Breckenridge and Kuksis, 1968). In our experiment, an increased supply and subsequent esterification of C16:0 at the *sn*-1 and *sn*-2 sites would potentially displace C14:0 and, to a lesser degree, FA from C8:0 to C12:0, effectively decreasing their secretion into milk (Figure 1). This competitive mechanism is likely to be predominant when long-chain SFA are fed, as opposed to any direct inhibition of lipogenic enzymes, such as that exhibited by specific FA such as *trans*-10,*cis*-12 C18:2 (Bauman et al., 2011).

The increase in both concentration and yield of pre-formed FA (>16-carbon) for SA, relative to PA, can in the same way be attributed to a greater availability of C18:0 for milk fat synthesis in the mammary gland with the SA treatment. However, compared with SA, PA increased the secretion of C16:0 (113 g/d) and its desaturation product, *cis*-9 C16:1 (4.5 g/d), into milk, whereas SA only modestly increased C18:0 secretion into milk (13 g/d) and did not have an effect on its desaturation product, *cis*-9 C18:1. These results would suggest that efficiency of incorporation of C16:0 into milk fat is greater than that of C18:0, which is in agreement with Enjalbert et al. (1998), whose results showed limited mammary uptake of C18:0, compared with C16:0. However, it is likely that other factors could have influenced the response, such as FA digestibility differences (not evaluated in this experiment) or a faster incorporation or utilization of C18:0 into other tissues, the latter leading to differential partitioning of C16:0 and C18:0 in tissues. Some indications exist that digestibility of supplemented C18:0 may be negatively affected, especially when it is fed at high doses (Steele, 1969) and its duodenal flow is increased. Ferlay et al. (1993) reported that digestibility of C18:0 decreased substantially, from 73 to 45%, when the duodenal flow of C18:0 increased from 170 to 445 g/d. However, it was recently shown that digestibility of C16:0 can also be decreased when fed at 2% of ration DM when compared with a non-added-fat diet (Piantoni et al., 2013). In our experiment, particle size of the C18:0-enriched fat supplement was slightly larger than that of the C16:0-enriched supplement, which could have limited the surface area available for digestion, decreasing its overall digestibility. Specific studies aimed to compare FA digestibility when feeding C16:0 and C18:0 relative to control, while taking particle size differences into account, are required to clarify potential differences in digestion between these FA.

CONCLUSIONS

Dietary supplementation with C16:0 improved milk fat concentration and yield as well as the efficiency of

feed conversion into milk compared with C18:0 supplementation. Further studies are required to investigate the effects of other dietary factors, such as fat, fiber, and starch content of the diet, on the response during early and established lactation. The determination of optimal dose to be fed under practical conditions is also required.

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