Milk production and nutrient digestibility responses to increasing levels of stearic acid supplementation of dairy cows

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ABSTRACT

The objective of our study was to evaluate the dose-response effects of a stearic acid (C18:0)-enriched supplement on nutrient digestibility, production responses, and the maximum amount of C18:0 that can be incorporated into the milk fat of dairy cows. Multiparous Holstein cows ($n = 32$; 145 ± 66 d in milk) with a wide range in milk yield (30 to 70 kg/d) were blocked by milk yield and assigned to replicated 4×4 Latin squares. Treatments were diets supplemented with a C18:0-enriched supplement (SA; 93% C18:0) at 0, 0.80, 1.50, or 2.30% of diet dry matter (DM). Periods were 21 d with the final 5 d used for data and sample collection. Dry matter intake increased linearly as SA supplementation increased. Supplementation of SA had no effect on the yield of milk or milk components. Due to the increase in DM intake, SA linearly reduced the ratio of energy-corrected milk to DM intake. Supplementation of SA did not affect body weight. Increasing SA reduced digestibility of 16-carbon, 18-carbon, and total fatty acids (FA), with the reduction in digestibility of 18-carbon FA being approximately 30 percentage units from the 0.0 to 2.30% SA supplemented diets. Supplementation of SA linearly increased concentrations of preformed milk fatty acids (FA) but did not affect the yield of preformed milk FA. Yields of C18:0 plus *cis*-9 C18:1 were increased by SA supplementation; however, the increase from 0 to 2.3% SA was only 16 g/d. The concentration and yield of de novo and 16-carbon milk FA were unaffected by SA supplementation. In conclusion, increasing doses of SA decreased FA digestibility and had little effect on production parameters. Although SA increased the yield of C18:0 and *cis*-9 C18:1 in milk fat, it had no overall effect on milk fat yield. The lack of production responses to a C18:0-enriched fat supplement was most likely associated with the marked decrease in FA digestibility.

Key words: stearic acid, dairy cow, milk fat, nutrient digestibility

INTRODUCTION

Fat supplements are commonly added to dairy cow diets to increase dietary energy density and yields of milk and milk fat and to improve energy balance (Rabiee et al., 2012). Although most commercially available fat supplements have typically contained mixtures of different fatty acids (FA), supplements enriched with individual FA are becoming increasingly available. Determining the effects of individual FA on nutrient digestibility, production responses, and metabolism of lactating dairy cows is therefore important. Much of the focus has been on individual SFA, particularly palmitic acid (C16:0; e.g., Piantoni et al., 2013; de Souza et al., 2016; Rico et al., 2017). There has been less focus on nutrient digestibility (Piantoni et al., 2015) and production (Steele and Moore, 1968; Steele, 1969; Piantoni et al., 2015) responses to stearic acid (C18:0), with all studies feeding only single or very high levels of C18:0. Because C18:0 is the main end product of biohydrogenation and the major FA available for absorption in the small intestine (Palmquist et al., 2005), it is often considered rumen inert and potentially an ideal FA to feed in a FA supplement for lactating dairy cattle. However, in our recent meta-analysis examining individual FA digestibility, we observed that increasing C18:0 flow through the duodenum had a strong negative effect on digestibilities of C18:0 and total FA (Boerman et al., 2015). This raises a question regarding the effect of C18:0-enriched supplements on total FA digestibility and performance of dairy cows. Hence, determining the dose-response effects of a C18:0-enriched supplement on nutrient digestibility and production responses of dairy cows is of particular interest.

Both C18:0 and *cis*-9 C18:1 are major FA in milk fat, with concentrations potentially limited by the requirement of the mammary gland to produce milk fat that is fluid at body temperature. The relatively high melting point of C18:0 requires the production of de novo synthesized FA and the conversion of C18:0 to *cis*-9 C18:1

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in the mammary gland to maintain fluidity (Palmquist, 2006). In a meta-analysis, Glasser et al. (2008) reported that desaturation of C18:0 in the mammary gland is positively and linearly related to mammary uptake of C18:0. Because there is an interdependence between de novo and 18-carbon milk FA for milk fat synthesis (Glasser et al., 2008), we are uncertain if C18:0 itself will affect the synthesis of de novo FA in the mammary gland. Therefore, it would be interesting to determine whether there is a maximum amount of C18:0 that can be incorporated into milk fat while not reducing de novo synthesis, thus maximizing milk fat yield.

Few studies have evaluated production responses to purified sources of C18:0 in dairy cows, and inconsistent results have been observed (Steele and Moore, 1968; Steele, 1969; Piantoni et al., 2015). Variability across experiments could be due to the use and feeding rate of different types of fat supplements, interaction with other dietary components, or the physiological status of cows. Feeding a relatively pure source of C18:0 at approximately 4% of diet DM resulted in an increase in milk fat yield with no effect on milk yield (Steele and Moore, 1968). However, in a subsequent study with a similar C18:0 inclusion level, an increase in milk yield was observed with no effect on milk fat concentration or yield (Steele, 1969). More recently, Piantoni et al. (2015) fed a C18:0-enriched supplement at 2% of diet DM and reported increased DMI, milk yield, and milk fat yield, with increases more evident in cows with higher milk yields, indicating that there was significant variation in response. Considering that most dairy farms that use supplemental fat would include it in diets within the range of 0.5 to 2.5% of ration DM, determining an optimal dose within this range has important applications. Therefore, the objective of our study was to evaluate the dose-response effects of a C18:0-enriched supplement on nutrient digestibility, production responses, and the maximum amount of C18:0 that can be incorporated into milk fat of dairy cows with a wide range of milk 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 mid-lactation (147 ± 66 DIM; mean $\pm$ SD) multiparous Holstein cows from the Michigan State University Dairy Field Laboratory were blocked by milk yield [46.6 ± 9.6 kg/d (mean $\pm$ SD), range 29.6 to 70.4 kg/d] and then randomly assigned to treatment sequence in a replicated 4×4 Latin square design balanced for carryover effects

with 21-d periods. All animals received a common diet with no fat supplementation during a 14-d preliminary period to obtain baseline values.

Treatments consisted of a control diet (**CON**) and 3 treatments supplemented with increasing doses of a stearic acid (C18:0)-enriched supplement (**SA**) at 0.80% (**L-SA**), 1.50% (**M-SA**), and 2.30% SA (**H-SA**). The SA supplement was a free FA product of high purity and contained approximately 6.60% C16:0, 93.0% C18:0, and 0.60% C22:0 (Wawasan Agrolipids Johor, Malaysia). Chemical composition and characteristics of the SA supplement are presented in Table 1. The ingredient and nutrient composition of the diets fed as TMR are described in Table 2. Minerals and vitamins were formulated to meet NRC (2001) recommendations. The SA supplement replaced soyhulls in the diet; replacing soyhulls would have less of an effect on production and metabolic responses, as well as on rumen function, compared with other feed ingredients. For example, we recently observed that the production response to a C16:0-enriched supplement was greater when it replaced soyhulls compared with when it replaced dry ground corn in the diet (de Souza et al., 2016). The DM concentration was determined twice weekly for forages, and diets were adjusted when necessary. All cows were housed in a tiestall barn throughout the experiment and milked twice daily (0430 and 1530 h). Access to feed was blocked from 1000 to 1200 h to allow for collecting orts and offering feed. Cows were fed 115% of expected intake at 1200 h daily. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and data for production results were collected during the last 5 d of each treatment period (d 17

Table 1. Characterization of the C18:0-enriched supplement fed during treatment periods

Item	Mean $\pm$ SD ¹
Particle size, μ m	659 $\pm$ 16.5
Melting point, $^{\circ}$ C	67.7
Iodine value	11.3
Free fatty acids (FA), %	98.0
Gross energy, Mcal/kg	9.41
FA profile, g/100 g of FA	
16:0	6.59 $\pm$ 0.01
17:0	0.14 $\pm$ 0.001
18:0	92.6 $\pm$ 0.02
<i>cis</i> -9 18:1	0.08 $\pm$ 0.001
22:0	0.59 $\pm$ 0.01

¹Mean $\pm$ SD from composited samples taken during each period (n = 4).

to 21). Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and composited by period for analysis. Milk yield was recorded and 2 milk samples were collected 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.

For digestibility estimates, fecal samples (~400 g) were collected every 15 h during the last 5 d of each period, resulting in 8 samples per cow per period and representing every 3 h of a 24-h period to account for diurnal variation. Feces were stored at −20°C until dried and composited on equal DM basis for each cow period. Body weight was measured on the last 2 d of each period after the morning milking, and 4 trained investigators determined BCS on a 5-point scale in 0.25 increments on the last day of each period (Wildman et al., 1982).

Table 2. Diet ingredients and nutrient composition of treatment diets¹

Item	Treatment ²			
	CON	L-SA	M-SA	H-SA
Ingredient, % of DM				
Corn silage	19.4	19.3	19.3	19.3
Alfalfa silage	14.4	14.4	14.4	14.4
Wheat straw	2.60	2.60	2.60	2.60
Corn grain, dry ground	30.3	30.3	30.3	30.3
Soybean meal, 48% CP	26.1	26.1	26.1	26.1
Soybean hulls	2.31	1.50	0.71	—
C18:0-enriched supplement	—	0.80	1.50	2.30
Vitamin and mineral mix ³	3.39	3.40	3.40	3.40
Limestone	0.80	0.80	0.80	0.80
Sodium bicarbonate	0.79	0.80	0.80	0.80
Nutrient composition, % of DM				
DM ⁴	57.4	57.4	57.5	57.5
NDF	25.3	25.1	24.9	24.8
CP	18.9	18.9	18.9	18.7
Starch	30.6	30.6	30.6	30.6
Fatty acids	2.44	3.20	3.95	4.70
16:0	0.34	0.39	0.44	0.48
18:0	0.07	0.78	1.49	2.19
<i>cis</i> -9 18:1	0.49	0.48	0.48	0.48
<i>cis</i> -9, <i>cis</i> -12 18:2	1.25	1.25	1.24	1.24
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.16	0.16	0.16	0.16

¹Experimental diets fed to 32 cows in a 4 × 4 Latin square design with 21-d periods.

²Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement.

³Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos (The Mosaic Co., Plymouth, MN), 3.9% magnesium oxide, 2% soybean oil, and <1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.

⁴Expressed as percent of as fed.

Sample Analysis

Diet ingredients, orts, and fecal samples 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-mm screen; Arthur H. Thomas, Philadelphia, PA). Diet ingredients, orts, and fecal samples were analyzed for NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), CP (AOAC International, 2000; method 990.03), and starch (Hall, 2009) by Cumberland Valley Analytical Services, Inc. (Hagerstown, MD.) The FA concentration of diet ingredients, orts, and fecal samples were determined as described by Lock et al. (2013). Indigestible NDF was used as an internal marker to estimate fecal output to determine apparent total-tract digestibility of nutrients (Cochran et al., 1986). Indigestible NDF was estimated as NDF after a 240-h in vitro fermentation (Goering and Van Soest, 1970) using rumen fluid from a nonlactating mature cow fed dry hay; flasks were re-inoculated at 120 h to ensure a viable microbial population. Particle size of the fat supplement was determined as described by de Souza et al. (2017). The fat supplement was analyzed for melting point (method Cc 1-25; AOCS, 2013), iodine value (method Cd 1d-92; AOCS, 2013), free FA (method Ca 5a-40; AOCS, 2013), and gross energy using bomb calorimeter by Eurofins Global Inc. (Des Moines, IA).

Individual milk samples were analyzed for fat, true protein, and lactose concentrations 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. Milk samples used for analysis of FA composition were composited by period based on milk fat yield. Milk lipids were extracted and FAME prepared according to our previously described methods (Lock et al., 2013). Yields of individual FA (g/d) in milk fat were calculated by 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 content and other milk lipid classes (Piantoni et al., 2013). The FA yield response (**FAYR**) to additional FA intake was calculated for total FA and for C18:0 plus *cis*-9 C18:1 using the following equation:

$$\text{FAYR (\%)} = \left[\frac{(\text{FA yield for SA-supplemented diet} - \text{FA yield for CON})}{(\text{FA intake for SA-supplemented diet} - \text{FA intake for CON})} \right] \times 100.$$

Statistical Analysis

Two cows were removed from analysis because they developed mastitis during 2 consecutive periods. Therefore, all results are from the 30 remaining cows. All data were analyzed using a mixed model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C(S)_{i(j)} + S_j + P_k + T_l + e_{ijkl}$$

where Y_{ijk} = the dependent variable, μ = the overall mean, $C(S)_{i(j)}$ = random effect of cow nested within square ($i = 1$ to 4), S_j = fixed effect of square ($j = 1$ to 8), P_k = fixed effect of period ($k = 1$ to 4), T_l = fixed effect of treatment ($l = 1$ to 4), and e_{ijkl} = residual error. The interactions between period and treatment, period and square, and square and treatments were initially included in the model but removed because they were not significant (all $P > 0.20$). Two preplanned contrasts were used to determine the linear and quadratic effects of increasing doses of C18:0. Treatment terms were declared significant at $P \leq 0.05$ and trends were declared at $P \leq 0.10$. All data are expressed as least squares means and standard error of the means, unless otherwise specified.

RESULTS

The SA supplement had a mean particle size of 659 μm and melting point of 67.7°C (Table 1). As expected,

substituting SA for soyhulls increased dietary FA and reduced NDF content with no differences in CP or starch (Table 2). Dietary C18:0 content was 0.07, 0.78, 1.49, and 2.19% of diet DM for CON, L-SA, M-SA, and H-SA, respectively. A more modest increase was observed in C16:0 content of 0.34, 0.39, 0.44, and 0.48% of diet DM for CON, L-SA, M-SA, and H-SA, respectively.

Increasing SA linearly increased DMI ($P = 0.01$) with no effect on milk yield ($P = 0.17$; Table 3). The L-SA and M-SA treatments reduced milk fat content compared with CON and H-SA (quadratic, $P = 0.04$). Also, increasing SA inclusion tended to linearly reduce milk protein yield ($P = 0.07$) and milk lactose yield ($P = 0.10$). However, SA had no effect on milk fat yield or milk protein content (both $P > 0.10$). Increasing SA inclusion decreased feed efficiency (ECM/DMI; linear, $P = 0.001$) due to the increase in DMI and no positive treatment effects on the yield of milk or milk components. In our 21-d treatment periods, we observed no effect of SA on BW ($P = 0.39$), but increasing SA tended to reduce BCS (quadratic, $P = 0.09$).

Intakes of 16-carbon, 18-carbon, and total FA linearly increased with increasing SA ($P < 0.001$; Table 4). Increasing SA tended to decrease DM digestibility (linear, $P = 0.06$) but had no effect on NDF digestibility ($P = 0.58$). Increasing SA reduced total FA digestibility (quadratic, $P < 0.001$; Table 4), which was most evident for 18-carbon FA with digestibility reduced by ~30 percentage units (quadratic, $P < 0.001$)

Table 3. Production responses for cows fed treatment diets (n = 30)

Variable	Treatment ¹				SEM	TRT ²	Contrast ³	
	CON	L-SA	M-SA	H-SA			Linear	Quadratic
DMI, kg/d	26.4	26.8	27.6	27.7	0.53	0.09	0.01	0.71
Yield, kg/d								
Milk	37.6	37.8	37.7	36.5	1.10	0.17	0.11	0.14
Fat	1.40	1.37	1.38	1.38	0.03	0.65	0.45	0.44
Protein	1.31	1.31	1.29	1.26	0.03	0.19	0.07	0.20
Lactose	1.75	1.77	1.75	1.71	0.05	0.26	0.10	0.25
3.5% FCM ⁴	38.9	38.6	38.6	38.2	0.92	0.56	0.19	0.89
ECM ⁵	39.8	39.5	39.5	38.9	0.93	0.46	0.14	0.66
Milk composition, %								
Fat	3.76	3.71	3.71	3.82	0.08	0.13	0.21	0.04
Protein	3.48	3.50	3.48	3.50	0.04	0.76	0.93	0.88
Lactose	4.65	4.64	4.61	4.62	0.04	0.44	0.18	0.73
BW, kg	743	744	740	741	13.1	0.39	0.27	0.71
BCS	3.48	3.44	3.43	3.44	0.07	0.21	0.13	0.09
ECM/DMI	1.51	1.47	1.43	1.40	0.03	0.03	0.001	0.76

¹Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).

²TRT is the P -value associated with treatment effect.

³Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

⁴3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

⁵ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)] (Tyrrell and Reid, 1965).

when comparing CON and H-SA (Table 4). The digestibility of 16-carbon FA was reduced by ~13 percentage units (quadratic, $P < 0.001$) when comparing CON and H-SA. We observed linear reductions in 16-carbon, 18-carbon, and total FA digestibilities with increasing intakes of 16-carbon, 18-carbon, and total FA, respectively (all $P < 0.01$; Figure 1). Also, increasing FA intake linearly increased the amount of FA in feces for all SA-supplemented treatments (Supplementary Figure S1; <https://doi.org/10.3168/jds.2016-12101>). The increase in 16-carbon FA intake was 55 g/d and the increase in 16-carbon FA absorbed was 23 g/d when comparing CON and H-SA treatments (Table 4). The increase in 18-carbon FA intake was 749 g/d and the increase in 18-carbon FA absorbed was 228 g/d when comparing CON and H-SA treatments. The FAYR for C18:0 and *cis*-9 C18:1 in milk fat to increasing SA was <3% for all SA treatments.

Milk FA are derived from 2 sources: <16-carbon FA from de novo synthesis in the mammary gland and >16-carbon FA originating from extraction from plasma. Mixed-source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. On a yield basis, increasing SA had no effect on the sources of FA in milk fat (all $P > 0.10$; Table 5). Although the yield of preformed FA was not affected by treatment, supplementation of SA increased the yield of C18:0 plus *cis*-9 C18:1 (linear, $P = 0.005$), resulting in a 16 g/d increase for H-SA compared with CON. However, increasing the amount of 18-carbon FA absorbed had a small effect on increasing 18-carbon FA in milk fat ($R^2 = 0.07$; $P = 0.01$; Supplementary Figure S2; <https://doi.org/10.3168/jds.2016-12101>).

The concentrations of de novo milk FA were elevated for L-SA and M-SA compared with CON and H-SA (Table 6; quadratic, $P = 0.01$). Although feeding SA linearly reduced *cis*-9 C16:1 concentration ($P = 0.003$), we observed no differences in the concentration of C16:0 ($P = 0.45$) and mixed-source milk FA ($P = 0.35$). Increasing SA increased the concentration of preformed milk FA (linear, $P = 0.01$) because of increased concentrations of C18:0 and *cis*-9 C18:1 (linear, $P < 0.001$).

DISCUSSION

Inconsistent responses to feeding SFA requires additional research with enriched FA supplements to identify the effects of specific FA on production responses of cows varying in milk yield to clarify when or if these supplements should be fed. Because C18:0 is the main end product of biohydrogenation (Palmquist et al., 2005), it could be an ideal FA to feed in a FA supplement for lactating dairy cows. However, because most studies in the literature on SFA supplementation were conducted using supplements with combinations of C16:0 and C18:0, the potential benefits of C18:0 need to be evaluated while avoiding the potential confounding effects of other FA in a supplement. Also, some of these inconsistent responses to feeding SFA may be associated with the level of the supplemental fat in the diet and the production level of the cows. Therefore, determining an optimal dose of a C18:0-enriched supplement for cows has important practical applications.

Few studies in the literature have evaluated production responses to C18:0-enriched supplements in dairy

Table 4. Apparent total-tract digestibility, intake of fatty acids (FA), and absorbed FA for cows fed treatment diets (n = 30)

Variable	Treatment ¹				SEM	TRT ²	Contrast ³	
	CON	L-SA	M-SA	H-SA			Linear	Quadratic
FA intake, g/d								
Total FA	662	945	1,181	1,472	23.5	<0.001	<0.001	0.84
16-carbon	97.2	117	133	152	2.58	<0.001	<0.001	0.96
18-carbon	533	795	1,013	1,282	20.8	<0.001	<0.001	0.81
Digestibility, %								
DM	72.8	72.2	71.9	71.8	0.40	0.10	0.06	0.19
NDF	41.1	42.0	41.6	41.9	0.79	0.58	0.36	0.57
Total FA	80.1	65.2	55.4	52.5	1.10	<0.001	<0.001	<0.001
16-carbon	77.7	71.4	66.5	64.5	0.72	<0.001	<0.001	<0.001
18-carbon	81.6	64.9	54.9	51.7	1.15	<0.001	<0.001	<0.001
Absorbed FA, g/d								
Total FA	530	616	654	773	19.5	<0.001	<0.001	0.34
16-carbon FA	75.5	83.5	88.4	98.0	2.07	<0.001	<0.001	0.51
18-carbon FA	435	516	556	663	17.1	<0.001	<0.001	0.34

¹Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).

²TRT is the P -value associated with treatment effect.

³Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

Figure 1. Digestibilities of 16-carbon, 18-carbon, and total fatty acids (FA) by intakes of 16-carbon, 18-carbon, and total FA for cows fed treatment diets ($n = 30$): (A) 16-carbon FA digestibility (%) = $90.2 - 0.17 \times 16\text{-carbon FA intake (g/d)}$ ($P < 0.01$; $R^2 = 0.37$); (B) 18-carbon FA digestibility (%) = $93.6 - 0.043 \times 18\text{-carbon FA intake (g/d)}$ ($P < 0.001$; $R^2 = 0.56$); and (C) total FA digestibility (%) = $90.1 - 0.036 \times \text{total FA intake (g/d)}$ ($P < 0.001$; $R^2 = 0.52$).

cows (Steele and Moore, 1968; Steele, 1969; Piantoni et al., 2015). Results are inconsistent regarding the effect of increasing dietary C18:0 on DMI. In our study, SA increased DMI in a similar manner to the overall treatment effect of C18:0 supplementation reported by Piantoni et al. (2015). The increase in DMI observed by Piantoni et al. (2015) was driven by high-producing

cows, whereas we did not observe any interaction between treatments and squares because cows were assigned to squares based on preliminary milk yield. In addition, the average production level was lower in the current study than that reported by Piantoni et al. (2015), even though the range in milk production was similar. We observed no effect of SA on milk yield or milk fat yield in high-producing cows, unlike Piantoni et al. (2015), who observed that a C18:0-enriched supplement increased the yield of milk and milk fat in high-producing cows in one period of a crossover design with no effect in the other period. Steele and Moore (1968) reported an increase in milk fat yield with no effect on milk yield when C18:0 was supplemented, whereas a subsequent study found no effect on milk fat yield but an increase in milk yield when C18:0 was supplemented (Steele, 1969). Recently, in a study comparing C16:0- and C18:0-enriched supplements, C16:0 increased milk fat yield compared with C18:0, suggesting a greater potential for supplements enriched with C16:0 to increase the yield of milk fat (Rico et al., 2014).

Digestibility of C18:0 may be negatively affected by increased C18:0 availability for absorption in the small intestine (Weisbjerg et al., 1992). In our recent meta-analysis evaluating the digestibility of individual FA using duodenally cannulated cows, we determined that as the amount of C18:0 reaching the duodenum increased, the digestibility of C18:0 linearly decreased across diets containing no supplemental fat and a range of fat supplements encompassing a wide range of C18:0 duodenal flows (Boerman et al., 2015). Because we did not use duodenally cannulated cows in the current study, we were unable to estimate C18:0 digestibility per se due to rumen biohydrogenation. Rather, utilizing feed to feces measurements, we combined all 18-carbon FA into a single digestibility estimate. We observed a reduction of approximately 30 percentage units in 18-carbon FA digestibility as C18:0 supplementation increased when comparing H-SA and CON. This resulted in a 4.3-percentage-unit reduction in 18-carbon FA digestibility for each 100 g of 18-carbon FA intake (Figure 1). Interestingly, when comparing the slopes of the regressions of our current study with the meta-regression by Boerman et al. (2015), we observed a similar magnitude in the linear reduction in 18-carbon FA digestibility with increasing intake of 18-carbon FA compared with the linear reduction in C18:0 digestibility with increasing C18:0 duodenal flow (4.9-percentage-unit reduction in C18:0 digestibility for each 100 g of C18:0 flow through the duodenum). Similarly, Piantoni et al. (2015) observed that feeding a 98% C18:0 supplement (2% of diet DM) reduced 18-carbon FA digestibility by approximately 24 percentage units, and

Table 5. Milk fatty acid (FA) yields for cows fed treatment diets (n = 30)

Item	Treatment ¹				SEM	TRT ²	Contrast ³	
	CON	L-SA	M-SA	H-SA			Linear	Quadratic
Summations, ⁴ g/d								
De novo	398	394	394	390	13.4	0.72	0.29	0.99
Both	484	471	469	476	17.0	0.33	0.32	0.12
Preformed	453	448	452	458	12.8	0.69	0.45	0.35
C18:0 + <i>cis</i> -9 C18:1	291	289	297	307	8.26	0.02	0.005	0.19
Individual FA, ⁵ g/d								
C4:0	36.7	36.1	36.9	37.5	1.37	0.42	0.23	0.96
C6:0	28.4	27.9	28.7	28.6	1.02	0.65	0.45	0.66
C8:0	18.9	18.7	19.1	18.8	0.66	0.81	0.88	0.94
C10:0	55.1	54.5	55.2	53.6	2.06	0.57	0.32	0.59
C12:0	70.1	69.6	69.2	66.9	2.65	0.15	0.04	0.38
C14:0	175	173	172	171	6.05	0.60	0.19	0.75
C16:0	461	448	448	454	16.4	0.34	0.40	0.11
<i>cis</i> -9 C16:1	23.0	22.7	21.6	21.6	0.97	0.07	0.01	0.80
C18:0	85.8	84.7	91.5	95.6	3.25	0.001	<0.001	0.16
<i>trans</i> -9 C 18:1	1.84	1.84	1.77	1.75	0.06	0.13	0.02	0.83
<i>trans</i> -10 C18:1	4.62	4.99	4.37	4.01	0.32	0.001	<0.001	0.03
<i>trans</i> -11 C18:1	6.17	5.92	5.82	5.62	0.32	0.06	0.01	0.87
<i>trans</i> -12 C18:1	3.09	3.01	2.87	2.75	0.13	0.001	<0.001	0.79
<i>cis</i> -9 C18:1	205	204	206	212	5.65	0.30	0.11	0.29
<i>cis</i> -11 C18:1	7.12	7.02	6.61	6.76	0.32	0.07	0.03	0.39
<i>cis</i> -9, <i>cis</i> -12 C18:2	39.2	39.2	37.9	36.6	1.41	0.001	<0.001	0.21
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	5.03	5.08	4.88	4.79	0.18	0.02	0.006	0.35
<i>cis</i> -9, <i>trans</i> -11 C18:2	3.91	3.78	3.55	3.30	0.17	<0.0001	<0.001	0.56

¹Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).

²TRT is the *P*-value associated with treatment effect.

³Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

⁴Summations; de novo = milk FA <16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA >16 carbons in length.

⁵A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.

postulated that the low digestibility of C18:0-enriched FA supplements could have resulted from the greater prill size of the supplement fed. This explanation likely does not support our results because the prill size of the FA supplement used in the current study was similar to those used by de Souza et al. (2017) for a C16:0-enriched supplement, in which prill size had minor effects on FA digestibility. However, we cannot eliminate the possibility of the physical characteristics (e.g., melting point) of the fat supplement affecting digestibility and therefore production results.

Our results are further supported by a recent study that observed that increasing the proportion of C18:0 to C16:0 in supplemental fat reduced total FA and OM digestibilities in dairy cows (Chamberlain and DePeters, 2017). These findings highlight that the FA profile of a fat supplement is an important factor affecting overall FA digestibility and consequently production responses of dairy cows. Although the exact mechanisms for the reduction in C18:0 or 18-carbon FA digestibility are unknown, potential causes have been suggested and they include limits in lysolecithin and competition for absorption sites of C18:0 (Drackley, 2000). Lysolecithin

acts as an amphiphile for C18:0, aiding with FA solubilization that is required for absorption (Freeman, 1969). If absorption sites are limited or there is increased competition for binding sites, FA digestibility may also be reduced. Further research is required to determine the mechanisms that reduce C18:0 absorption and how to overcome it.

Using duodenal infusions of C16:0 and C18:0, Enjalbert et al. (2000) reported an apparent FAYR of 46.7% for C16:0 and 12.0% for C18:0. We calculated FAYR to determine the efficiency of utilization of the SA supplement (increased dietary C18:0) for milk fat yield in our current study. Both C18:0 and *cis*-9 C18:1 are included in the transfer efficiency calculations to account for changes that occur in the mammary gland via Δ^9 -desaturase. Increasing dietary C18:0 and *cis*-9 C18:0 by 100 g/d for the H-SA treatment resulted in a 2.1 g/d increase in C18:0 plus *cis*-9 C18:0 in milk fat, or a FAYR of 2.1%. The H-SA treatment had the highest FAYR, with FAYR for the L-SA and M-SA treatments both <1%. Piantoni et al. (2015) reported a FAYR for C18:0 plus *cis*-9 C18:0 of 8.2% from diet into milk fat. Compared with our results (<3% FAYR for all

treatments), the transfer efficiency from diet to milk fat is greater for diets supplemented with C16:0-enriched supplements (16.5 to 24% FAYR; Lock et al., 2013; Piantoni et al., 2013).

Piantoni et al. (2015) reported overall improvements in milk fat yield with an increase in preformed FA and 18:0 in milk fat when C18:0 was supplemented at 2% of diet DM. Although they observed an increase of 44 g/d in preformed FA in milk fat, we observed no effect of SA on the yield of preformed milk FA. The increase in preformed milk FA that Piantoni et al. (2015) reported came from a combination of C18:0 (20 g/d) and *cis*-9 C18:1 (21 g/d), whereas we observed a 16 g/d increase in C18:0 and *cis*-9 C18:1. Feeding a mostly saturated FA from prilled hydrogenated free FA (from tallow) containing a mixture of C16:0 and C18:0 resulted in an increase in milk fat yield of 177 g/d compared with a control diet (Relling and Reynolds, 2007). Comparing our results of no increase in milk fat to SA supplementation with studies that supplemented C16:0, the increase in milk FA ranged from 73 to 88 g/d when C16:0-enriched supplements were fed (Lock et al., 2013; Piantoni et al., 2013). Rico et al. (2014) compared a

C16:0-enriched with a C18:0-enriched supplement and observed a 90 g/d increase in total milk FA with the C16:0-enriched supplement due to a 117 g/d increase in 16-carbon FA with small reductions in both de novo and preformed milk FA. Therefore, the increase in milk fat yield observed when fat supplements containing a mixture of C16:0 and C18:0 are fed is most likely a result of the supplemental C16:0 rather than C18:0.

Besides differences in intestinal digestibility, a potential reason for the discrepancy between C16:0 and C18:0 transfer into milk fat is the difference in the incorporation of these 2 FA into milk fat triglycerides in the mammary gland. Fairly uniform distribution is observed for C16:0 on the *sn*-1 and *sn*-2 positions of the triglyceride, with more modest concentrations found on *sn*-3 (Jensen, 2002). However, C18:0 is preferentially located on the *sn*-1 position. Although found at all positions, *cis*-9 C18:1 is preferentially located on either the *sn*-1 or *sn*-3 position of the triglyceride (Jensen, 2002). Palmitoyl-CoA was 8 to 10 times more efficient for the initial acylation to glycerol 3-phosphate for triglyceride synthesis than stearoyl-CoA (Kinsella and Gross, 1973). This is thought to be because palmitoyl-CoA

Table 6. Milk fatty acid (FA) concentrations for cows fed treatment diets (n = 30)

Item	Treatment ¹				SEM	TRT ²	Contrast ³	
	CON	L-SA	M-SA	H-SA			Linear	Quadratic
Summations, ⁴ g/100 g of FA								
De novo	29.7	30.0	30.0	29.4	0.31	0.03	0.13	0.01
Mixed	36.0	35.7	35.5	35.6	0.45	0.35	0.15	0.29
Preformed	34.3	34.3	34.5	35.0	0.41	0.02	0.01	0.16
C18:0 + <i>cis</i> -9 C18:1	22.1	22.2	22.8	23.6	0.37	<0.001	<0.001	0.05
Individual FA, ⁵ g/100 g of FA								
C4:0	2.74	2.73	2.79	2.82	0.05	0.01	0.001	0.60
C6:0	2.11	2.12	2.17	2.16	0.03	0.04	0.02	0.45
C8:0	1.41	1.42	1.45	1.42	0.02	0.16	0.34	0.10
C10:0	4.12	4.16	4.19	4.05	0.08	0.07	0.29	0.02
C12:0	5.24	5.32	5.27	5.06	0.12	0.002	0.01	0.01
C14:0	13.1	13.2	13.1	12.9	0.15	0.01	0.01	0.01
C16:0	34.2	34.0	33.9	34.0	0.44	0.45	0.30	0.22
<i>cis</i> -9 C16:1	1.71	1.73	1.64	1.62	0.05	0.01	0.003	0.58
C18:0	6.53	6.50	6.99	7.35	0.18	<0.001	<0.001	0.07
<i>trans</i> -9 C 18:1	0.14	0.14	0.14	0.13	0.003	0.02	0.01	0.43
<i>trans</i> -10 C18:1	0.35	0.38	0.33	0.30	0.02	0.003	0.01	0.02
<i>trans</i> -11 C18:1	0.46	0.45	0.44	0.43	0.02	0.04	0.004	0.95
<i>trans</i> -12 C18:1	0.23	0.23	0.22	0.21	0.01	<0.001	<0.001	0.45
<i>cis</i> -9 C18:1	15.6	15.7	15.8	16.2	0.26	<0.001	<0.001	0.14
<i>cis</i> -11 C18:1	0.54	0.53	0.50	0.51	0.02	0.01	0.005	0.39
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.96	2.98	2.89	2.77	0.05	<0.001	<0.001	0.01
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.38	0.39	0.37	0.36	0.01	<0.001	<0.001	0.05
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.30	0.29	0.27	0.25	0.01	<0.001	<0.001	0.26

¹Treatments contained 0.0% (CON), 0.8% (L-SA), 1.5% (M-SA), or 2.3% (H-SA) C18:0-enriched fat supplement (SA).

²TRT is the *P*-value associated with treatment effect.

³Contrasts correspond to the linear effect of increasing SA and the quadratic effect of increasing SA.

⁴Summations; de novo = milk FA <16 carbons in length; mixed = milk FA 16-carbons in length; preformed = milk FA >16 carbons in length.

⁵A total of approximately 70 individual FA were quantified and used for calculations (summation by source). Only select FA are reported in the table.

accelerates the incorporation of de novo synthesized FA to the other positions of the triglyceride. As C18:0 uptake by the mammary gland increases, desaturation of C18:0 to *cis*-9 C18:1 increases to maintain fluidity (Enjalbert et al., 1998). *Cis*-9 C18:1 reduces total de novo synthesis, potentially reducing total milk FA yield by competing with butyryl-CoA for attachment at the *sn*-3 position of the triglyceride (Hansen and Knudsen, 1987). However, in our study, we observed no effect of supplemental SA on de novo synthesized milk FA, probably because of the modest increases in C18:0 and *cis*-9 C18:1 in milk fat.

CONCLUSIONS

Increasing C18:0 supplementation increased DMI but did not affect yields of milk or milk components, and therefore reduced ECM/DMI. Increasing C18:0 supplementation reduced digestibilities of 16-carbon, 18-carbon, and total FA. Supplementing C18:0 resulted in a modest increase in C18:0 and *cis*-9 C18:1 FA in milk fat, but the increase was not enough to positively affect milk fat yield. The lack of production responses was most likely associated with the marked decrease in FA digestibility with increasing C18:0 supplementation.

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