Altering the ratio of dietary C16:0 and *cis*-9 C18:1 interacts with production level in dairy cows: Effects on production responses and energy partitioning

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ABSTRACT

The objective of our study was to evaluate the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids on nutrient digestibility, energy partitioning, and production responses of lactating dairy cows. Cows were blocked by milk yield and assigned to 3 groups (12 cows per group) in a main plot: low (45.2 ± 1.7 kg/d), medium (53.0 ± 1.6 kg/d), and high (60.0 ± 1.9 kg/d). Within each production group, a truncated Latin square arrangement of fatty acid (FA) treatments was used in 2 consecutive 35-d periods. The FA treatments supplemented at 1.5% of diet dry matter were (1) 80:10 (80% C16:0 + 10% *cis*-9 C18:1), (2) 73:17 (73% C16:0 + 17% *cis*-9 C18:1), (3) 66:24 (66% C16:0 + 24% *cis*-9 C18:1), and (4) 60:30 (60% C16:0 + 30% *cis*-9 C18:1). Treatment $\times$ production group interactions were observed for yields of milk, fat-corrected milk, energy-corrected milk, milk fat, milk protein, and milk lactose and energy partitioned to milk. Increasing *cis*-9 C18:1 in FA treatments reduced fat-corrected milk, energy-corrected milk, and milk energy output in low-producing cows but increased these in high-producing cows. Increasing *cis*-9 C18:1 in FA treatments did not affect milk yield, milk protein yield, and milk lactose yield in low- and medium-producing cows but increased these in high-producing cows. Regardless of production level, there was no effect of treatments on dry matter intake; however, increasing *cis*-9 C18:1 in FA treatments increased body weight change and body condition score change. Increasing *cis*-9 C18:1 in FA treatments increased total FA digestibility due to a linear increase in 16- and 18-carbon FA digestibilities. Interactions between FA treatments and production level were observed for the yield of milk fat and milk FA sources. In low-producing cows, increas-

ing *cis*-9 C18:1 in FA treatments decreased milk fat yield due to a decrease in de novo and mixed milk FA without changes in preformed milk FA. In contrast, in high-producing cows, increasing *cis*-9 C18:1 in FA treatments increased milk fat yield due to an increase in de novo and preformed milk FA. Our results indicate that high-producing dairy cows (averaging 60 kg/d) responded better to a fat supplement containing more *cis*-9 C18:1, whereas low-producing cows (averaging 45 kg/d) responded better to a supplement containing more C16:0.

Key words: palmitic acid, oleic acid, nutrient partitioning, production level

INTRODUCTION

The addition of supplemental fat sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production and recovery of body condition. Although in general fat supplementation has been shown to increase milk yield, the efficiency of milk production, and reproductive performance, great variation has been reported for different fat types and for the same supplement across different diets and studies (Rabiee et al., 2012; Rodney et al., 2015). Understanding the effects of different fatty acid (FA) sources on milk production and energy partitioning is crucial, and attention has lately been given to determining the effects of specific individual FA. However, to our knowledge, few studies were designed to evaluate the effects of different FA ratios on the production responses of dairy cows. Recently, we observed that compared with a nonfat control diet, feeding an FA blend with a high content of palmitic (C16:0) acid (80% C16:0) increased milk energy output and energy partitioning toward milk, whereas feeding an FA blend with a combination of palmitic C16:0 and oleic (*cis*-9 C18:1) acids (45% C16:0 and 35% *cis*-9 C18:1) increased energy allocated to BW and energy partitioning to BW (de Souza et al., 2018). These results suggest that C16:0 and *cis*-9 C18:1 are able to alter nutrient partitioning between the mammary gland

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and adipose tissue, which may allow for different FA supplements to be used in different situations according to the metabolic priority of dairy cows and management needs. Unfortunately, only 1 combination of C16:0 and *cis*-9 C18:1 was evaluated by de Souza et al. (2018); therefore, determining the effect of other dietary ratios of these FA is of particular importance.

Additionally, reasons for variability across experiments for different FA supplements could be associated with physiological state of cows. Production level is well established as a potential factor that interacts with nutrition, affecting production responses of dairy cows (e.g., Harvatine and Allen, 2005; Piantoni et al., 2015). Palmquist and Jenkins (1980) reported that low-producing cows did not respond to fat supplementation compared with high-producing cows in their feeding trials. Supplementation with C16:0 has been shown to increase milk fat yield regardless of level of milk production (Piantoni et al., 2013; Rico et al., 2014b). In contrast, stearic acid (C18:0) supplementation increased DMI and milk production in higher-producing cows but not in lower-producing cows (Piantoni et al., 2015). Although reasons for the interaction between C18:0 and production level were not determined, these results suggest that high-producing cows may respond better to supplements containing 18-carbon FA. Although it is well established that high-producing cows have greater energy demands, resulting in changes in DMI and microbial fermentation, other differences in partitioning of absorbed FA between milk and body reserves are also possible.

Therefore, the objective of our study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in cows with different production levels on nutrient digestibility, energy partitioning, and production responses of lactating dairy cows. We hypothesized that increasing the amount of *cis*-9 C18:1 in supplemental fat would increase energy partitioning to body reserves and that feeding *cis*-9 C18:1 would increase milk yield in high-producing cows but not in low-producing cows.

MATERIALS AND METHODS

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. This trial was designed to test the interaction between production level and feeding fat supplements varying in the ratios of C16:0 and *cis*-9 C18:1. Thirty-six mid-lactation multiparous Holstein cows from the Michigan State University Dairy Field Laboratory (East Lansing) were used in a split-plot truncated balanced Latin square design. All animals

received a common diet with no fat supplementation during a 14-d preliminary period to obtain baseline values. Cows were blocked in 3 groups of 12 cows each by production level based on the data collected during the preliminary period (Table 1). Groups were (1) low-producing cows (milk yield = 45.2 ± 1.7 kg/d; 115 ± 42 DIM), (2) medium-producing cows (milk yield = 53.0 ± 1.6 kg/d; 105 ± 41 DIM), and (3) high-producing cows (milk yield = 60.0 ± 1.9 kg/d; 104 ± 43 DIM). There were no differences in the average genomic total performance index of cows across production groups. Within each production level group, a 4×2 balanced truncated Latin square design was used in 2 consecutive 35-d periods. The 4 treatments were combinations of 2 commercially available FA supplements (palmitic acid-enriched free FA prills and Ca salts of palm FA) that differed in FA profile that were blended to achieve different ratios of C16:0 and *cis*-9 C18:1 in the FA supplement blends (Table 2). The FA treatments were (1) 80% C16:0 + 10% *cis*-9 C18:1 (**80:10**), (2) 73% C16:0 + 17% *cis*-9 C18:1 (**73:17**), (3) 66% C16:0 + 24% *cis*-9 C18:1 (**66:24**), and (4) 60% C16:0 + 30% *cis*-9 C18:1 (**60:30**). The FA supplement blends provided 1.5% FA (% of diet DM). The ingredient and nutrient compositions of the diets fed as TMR are described in Table 3. Truncated Latin square designs have been used previously in experiments in dairy science (e.g., Clark and Armentano, 1999; Weiss et al., 2013). For our truncated Latin square design with 4×2 treatments and periods, multiples of 12 cows per production group were required to completely balance treatment sequences within block; therefore, each cow was assigned to 2 treatments in 2 different periods. We used this truncated design to allow longer experimental periods for data measurements compared with more traditional 2- to 3-wk periods for complete Latin squares.

Dry matter concentration of forages was determined twice weekly, and diets were adjusted when necessary. Throughout the experiment, cows were housed in individual tiestalls. Access to feed was blocked daily from 0830 to 1000 h to allow for the collection of orts and feed delivery. Cows were fed 115% of expected daily intake, and feed intake was recorded daily. Water was available ad libitum in each stall, and stalls were bedded with sawdust and cleaned twice per day.

Data and Sample Collection

Samples and data for production variables, digestibility, and plasma metabolites and hormones were collected during the last 5 d of each treatment period (d 31–35). Samples (0.5 kg) of all diet ingredients and orts (12.5%) from each cow were collected daily and composited by period for analysis. Fecal (~400 g) and blood (~15 mL)

Table 1. Baseline data for cows used in this study, obtained during the preliminary period when cows were fed a common diet (mean $\pm$ SD)

Parameter	Production level		
	Low	Medium	High
Milk yield, kg	45.2 $\pm$ 1.7	53.0 $\pm$ 1.6	60.0 $\pm$ 1.9
Fat content, %	3.53 $\pm$ 0.18	3.43 $\pm$ 0.19	3.31 $\pm$ 0.20
Protein content, %	3.15 $\pm$ 0.15	3.12 $\pm$ 0.13	3.08 $\pm$ 0.16
Lactose content, %	4.84 $\pm$ 0.10	4.82 $\pm$ 0.09	4.80 $\pm$ 0.11
Fat yield, kg	1.59 $\pm$ 0.12	1.77 $\pm$ 0.13	1.98 $\pm$ 0.15
Protein yield, kg	1.42 $\pm$ 0.12	1.65 $\pm$ 0.18	1.84 $\pm$ 0.20
Lactose yield, kg	2.17 $\pm$ 0.23	2.55 $\pm$ 0.25	2.88 $\pm$ 0.28
BW, kg	688 $\pm$ 73	651 $\pm$ 51	700 $\pm$ 63
BCS	3.18 $\pm$ 0.38	3.17 $\pm$ 0.30	3.13 $\pm$ 0.35
DIM	115 $\pm$ 42	105 $\pm$ 41	104 $\pm$ 43

samples were collected every 15 h during the last 5 d of each period, totaling 8 samples per cow per period. The 15-h interval over 5 d simulates sampling every 3 h over a 24-h period. Feces were stored in a sealed plastic cup at -20°C . Blood was stored on ice until centrifugation at $2,000 \times g$ for 15 min at 4°C (within 30 min of sample collection). Plasma was transferred into microcentrifuge tubes and stored at -20°C until composited by cow and period. 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 being analyzed for FA composition.

Body weight measurements were taken 3 times per week following the afternoon milking. On the last day of the preliminary period and last day of each treat-

ment period, 3 trained investigators determined BCS on a 5-point scale in 0.25-point increments (Wildman et al., 1982).

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). Feed ingredients, Orts, and feces were analyzed for NDF, CP, starch, and FA concentration as described by Boerman et al. (2017). Gross energy of feed ingredients, Orts, and feces were determined by bomb calorimetry (Parr Instrument Inc., Moline, IL). 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

Table 2. Proportion of each fatty acid (FA) supplement for treatment blends and FA profile of FA blends

Item	Treatment ¹			
	80:10	73:17	66:24	60:30
FA supplement in treatment blends, %				
C16:0-enriched FA supplement ²	89.0	66.5	45.5	29.0
Ca salts of palm FA supplement ³	11.0	33.5	54.5	71.0
FA profile of each FA blend, g/100 g of FA				
C14:0	0.67	0.75	0.82	0.88
C16:0	80.7	73.6	66.3	59.7
C18:0	1.83	1.79	1.75	1.70
<i>cis</i> -9 C18:1	10.2	17.5	23.9	29.7
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.95	4.23	4.45	5.55
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.11	0.15	0.19	0.23

¹80:10 = 1.5% of FA supplement blend to provide $\sim$ 80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide $\sim$ 73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide $\sim$ 66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide $\sim$ 60% C16:0 and 30% *cis*-9 C18:1.

²Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). Contained (g/100 g of FA) 0.60 of C14:0, 84.5 of C16:0, 1.80 of C18:0, and 7.90 of *cis*-9 C18:1, and 98.8% total FA.

³Calcium salts of palm FA supplement (Nutracal; Wawasan Agrolipids). Contained (g/100 g of FA) 1.0 of C14:0, 48.1 of C16:0, 1.12 of C18:0, and 39.9 of *cis*-9 C18:1, and 83.4% total FA.

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. Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990; method 972.160; Universal Lab Services, Lansing, MI). Yields of 3.5% FCM, ECM, milk energy, and milk components were calculated using milk yield and component concentrations from each milking, summed for a daily total, and averaged for each collection period. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 31–35 of each period). Milk lipids were extracted, and FAME were prepared and quantified using GLC according to Lock et al. (2013). Yield of individual FA (g/d) in milk fat was 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 content and other milk lipid classes (Piantoni et al., 2013).

Energy Partitioning Calculations

We determined energy partitioning using the procedures described by de Souza and Lock (2018). Energy partitioning was determined during treatment periods using weekly milk samples taken twice a week and analyzed for fat, protein, and lactose concentrations. Body weight measurements were taken 3 times per week following the afternoon milking, and BCS was determined by 3 trained investigators on the last day of each period. Data were used to calculate milk energy output, metabolic BW, and body tissue gain throughout treatment periods.

Energy intake was calculated according to Harvatine and Allen (2006a) using the NRC (2001) equation digestible energy intake = gross energy intake $\times$ gross energy digestibility; NE_L intake was calculated from digestible energy according to NRC (2001). Energy output was calculated according to Hardie et al. (2015).

Table 3. Ingredient and nutrient composition of the treatment diets

Item	Treatment ¹			
	80:10	73:17	66:24	60:30
Ingredient, % of DM				
Corn silage	25.5	25.5	25.5	25.5
Alfalfa silage	16.3	16.3	16.3	16.3
Wheat straw	5.32	5.32	5.32	5.32
Ground corn	15.9	15.9	15.9	15.9
High-moisture corn	14.2	14.2	14.2	14.2
Soybean meal	12.1	12.1	12.1	12.1
Soyhulls	4.82	4.76	4.70	4.65
Protein supplement ²	1.09	1.09	1.09	1.09
C16:0-enriched FA supplement ³	1.37	1.06	0.76	0.48
Ca salts of palm FA supplement ⁴	0.17	0.54	0.90	1.23
Mineral and vitamin mix ⁵	3.23	3.23	3.23	3.23
Nutrient composition, ⁶ % of DM				
NDF	29.0	29.0	29.0	29.0
CP	16.5	16.5	16.5	16.5
Starch	28.8	28.8	28.8	28.8
FA	4.00	3.98	4.00	3.98
16:0	1.58	1.44	1.33	1.26
18:0	0.05	0.04	0.04	0.04
<i>cis</i> -9 18:1	0.68	0.78	0.88	0.98
<i>cis</i> -9, <i>cis</i> -12 18:2	1.25	1.25	1.27	1.29
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.20	0.20	0.20	0.20

¹80:10 = 1.5% of fatty acid (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²Protein supplement (Perdue Agribusiness, Salisbury, MD).

³Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). Contained (g/100 g of FA) 0.60 of C14:0, 84.5 of C16:0, 1.80 of C18:0, and 7.90 of *cis*-9 C18:1, and 98.8% total FA.

⁴Calcium salts of palm FA supplement (Nutracal; Wawasan Agrolipids). Contained (g/100 g of FA) 1.0 of C14:0, 48.1 of C16:0, 1.12 of C18:0, and 39.9 of *cis*-9 C18:1, and 83.4% total FA.

⁵Contained 34.1% dry ground shelled 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.

⁶Expressed as a percentage of as fed.

Milk energy output was calculated according to NRC (2001) as milk energy output (Mcal/d) = $9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}$. Mean daily BW change (kg/d) was calculated for each cow within period by linear regression after 2 iterations of removing outliers. Energy output in body reserves was estimated according to NRC (2001) as body reserves (Mcal/d) = $(2.88 + 1.036 \times \text{BCS}) \times \Delta\text{BW}$, where BCS is the average BCS for the study and ΔBW is BW change. Energy for maintenance (Mcal/d) was the difference between the energy intake and energy allocated to milk and BW.

Statistical Analysis

All data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + F_i + c_j(F_i) + P_k + T_l + F_i \times T_l + P_k \times T_l + P_k \times F_i + F_i \times P_k \times T_l + e_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, F_i = fixed effect of production level ($i = 3$), $c_j(F_i)$ = random effect of cow within the main plot ($j = 1$ to 12), P_k = fixed effect of period ($k = 1$ to 2), T_l = fixed effect of FA treatment ($l = 1$ to 4), and e_{ijkl} = 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 tendencies were declared at $0.05 < P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$, and tendencies were declared at $0.10 < P \leq 0.15$. Interactions between treatment and period and between production level and period were evaluated but removed from the statistical model when not significant ($P > 0.20$). Orthogonal contrasts were used to test the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion for the main effect of FA treatment and the interaction between production level and FA treatments.

RESULTS

Nutrient Intake and Total-Tract Digestibility

The FA treatments did not affect intakes of DM ($P = 0.68$; Table 4), NDF ($P = 0.66$), and total FA ($P = 0.70$) or the digestibility of DM ($P = 0.17$) or NDF ($P = 0.58$). Increasing *cis*-9 C18:1 in FA treatments decreased intake of 16-carbon FA (linear, $P < 0.01$) and increased the intake of 18-carbon FA (linear, $P < 0.01$). Increasing *cis*-9 C18:1 in FA treatments increased total FA digestibility (linear, $P = 0.02$), 16-carbon FA digestibility (linear, $P < 0.01$), and 18-carbon FA digestibility (quadratic, $P = 0.05$). Increasing *cis*-9 C18:1 in FA treatments decreased absorbed 16-carbon FA (quadratic, $P = 0.03$) and increased absorbed 18-carbon FA (linear, $P < 0.01$). The interaction between FA

Table 4. Nutrient intake and nutrient digestibility for cows fed treatment diets ($n = 36$)

Variable	Treatment ¹				SEM	P-value ²			Contrast ³		
	80:10	73:17	66:24	60:30		Trt	Production	Trt $\times$ production	Linear	Quadratic	Cubic
Intake, kg/d											
DMI	29.1	29.0	28.9	29.3	0.52	0.68	<0.01	0.89	0.82	0.88	0.89
NDF	8.43	8.42	8.40	8.35	0.16	0.66	0.01	0.92	0.64	0.85	0.98
Intake, g/d											
Total FA	1,164	1,148	1,132	1,142	23.0	0.70	0.01	0.81	0.36	0.50	0.78
16-carbon FA	464	425	379	370	8.92	<0.01	0.03	0.73	<0.01	0.06	0.19
18-carbon FA	644	661	693	721	13.4	<0.01	0.01	0.86	<0.01	0.62	0.71
Digestibility, %											
DM	66.2	66.7	67.0	67.3	0.66	0.17	0.21	0.18	0.10	0.71	0.97
NDF	40.2	39.0	40.0	40.9	0.99	0.58	0.60	0.24	0.90	0.27	0.39
Total FA	76.5	80.1	79.0	80.6	1.10	0.04	0.43	0.17	0.02	0.32	0.13
16-carbon FA	72.9	76.8	75.2	78.1	1.07	<0.01	0.22	0.16	<0.01	0.62	0.12
18-carbon FA	79.8	83.5	82.8	82.7	1.11	0.04	0.52	0.18	0.06	0.05	0.22
Absorbed, g/d											
Total FA	896	921	896	924	16.4	0.23	<0.01	0.50	0.09	0.50	0.13
16-carbon FA	337	326	285	287	6.98	<0.01	0.01	0.33	<0.01	0.03	0.12
18-carbon FA	518	551	573	595	9.63	<0.01	<0.01	0.47	<0.01	0.49	0.75

¹80:10 = 1.5% of fatty acid (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²P-values associated with treatment (Trt), production level, and interaction.

³Contrast refers to the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

Table 5. Milk yield, milk composition, BW, and BCS of cows fed the treatment diets (n = 36)

Variable	Treatment ¹				SEM	P-value ²			Contrast ³		
	80:10	73:17	66:24	60:30		Trt	Production	Trt × production	Linear	Quadratic	Cubic
Milk yield, kg/d											
Milk	48.1	49.5	49.4	50.3	1.11	0.09	<0.01	0.09	0.08	0.14	0.52
3.5% FCM ⁴	49.6	49.8	50.0	50.1	1.16	0.97	<0.01	0.05	0.65	0.98	0.99
ECM ⁵	49.6	50.1	50.2	50.6	1.15	0.87	<0.01	0.05	0.42	0.97	0.88
Milk composition											
Fat, kg/d	1.78	1.75	1.76	1.74	0.05	0.92	0.02	0.08	0.62	0.85	0.68
Fat, %	3.71	3.57	3.57	3.48	0.09	0.04	0.27	0.47	<0.01	0.59	0.31
Protein, kg/d	1.53	1.58	1.58	1.62	0.05	0.07	<0.01	0.03	0.06	0.91	0.51
Protein, %	3.18	3.21	3.21	3.24	0.05	0.47	0.02	0.42	0.16	0.87	0.51
Lactose, kg/d	2.15	2.24	2.25	2.30	0.06	0.05	<0.01	0.05	0.03	0.71	0.53
Lactose, %	4.47	4.54	4.55	4.57	0.03	0.05	0.05	0.08	<0.01	0.47	0.53
FCM/DMI	1.71	1.71	1.72	1.73	0.03	0.95	<0.01	0.04	0.58	0.95	0.98
BW, kg	710	705	704	709	10.2	0.25	0.06	0.66	0.74	0.05	0.65
BW change, kg/d	0.50	0.84	0.96	0.84	0.09	0.01	0.74	0.61	0.01	0.02	0.97
BCS	3.31	3.36	3.38	3.35	0.05	0.63	0.14	0.13	0.46	0.29	0.95
BCS change	0.08	0.15	0.22	0.28	0.04	<0.01	0.25	0.76	<0.01	0.87	0.79

¹80:10 = 1.5% of fatty acid (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²P-values associated with treatment (Trt), production level, and interaction.

³Contrast refers to the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

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).

treatments and production level was not significant for nutrient intake and digestibility variables (Table 4).

Production Responses

Increasing *cis*-9 C18:1 in FA treatments tended to increase milk yield (linear, $P = 0.08$; Table 5). Although increasing *cis*-9 C18:1 in FA treatments decreased milk fat content (linear, $P < 0.01$), we did not observe treatment differences for 3.5% FCM ($P = 0.97$), ECM ($P = 0.87$), or milk fat yield ($P = 0.92$). Increasing *cis*-9 C18:1 in FA treatments increased milk lactose content (linear, $P < 0.01$) and yield (linear, $P = 0.03$) and tended to increase milk protein yield (linear, $P = 0.06$). Increasing dietary *cis*-9 C18:1 in FA treatments also increased BW change (quadratic, $P = 0.02$) and BCS change (linear, $P < 0.01$).

We observed an interaction between FA treatments and production level for milk yield ($P = 0.09$; Table 5) due to increasing dietary *cis*-9 C18:1 in FA treatments increasing milk yield in high-producing cows but not in low- and medium-producing cows (linear, $P < 0.05$; Figure 1A). We also observed an interaction between FA treatments and production level for 3.5% FCM ($P = 0.05$), ECM ($P = 0.05$), and fat yield ($P = 0.08$) due to increasing *cis*-9 C18:1 in FA treatments increasing these variables in high-producing cows but decreasing these variables in low-producing cows (linear, $P <$

0.05; Figures 1B–D). Also, increasing *cis*-9 C18:1 in FA treatments increased the yields of milk protein and lactose in high-producing cows but not in low- and medium-producing cows (linear interaction; $P < 0.05$; Figure 1E–F).

Milk FA Concentration and Yield

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. Increasing *cis*-9 C18:1 in FA treatments decreased the concentration of mixed milk FA (linear, $P < 0.01$; Table 6) and increased the concentration of preformed milk FA (linear, $P < 0.01$). These results were due to increasing *cis*-9 C18:1 in FA treatments decreasing the concentration of C16:0 (linear, $P < 0.01$; Supplemental Table S1, <https://doi.org/10.3168/jds.2019-16374>) while increasing several *trans* milk FA (linear, all $P < 0.05$) and *cis*-9 C18:1 (quadratic, $P = 0.02$). On a yield basis, increasing *cis*-9 C18:1 in FA treatments increased the yield of preformed FA (linear, $P < 0.01$) due to an increase in *cis*-9 C18:1 and several *trans* milk FA (linear, all $P < 0.05$; Supplemental Table S2, <https://doi.org/10.3168/jds.2019-16374>). Although there was no effect of treatment on the yield

Figure 1. Effects of varying the dietary ratio of palmitic and oleic acids on (A) milk yield, (B) 3.5% FCM, (C) ECM, (D) milk fat yield, (E) milk protein yield, and (F) milk lactose yield of dairy cows across different production levels (low = 45.2 ± 1.7 kg/d; medium = 53.0 ± 1.6 kg/d; high = 60.0 ± 1.9 kg/d). Treatments were as follows: 80:10 = 1.5% of fatty acid (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between FA treatments and production level were detected for milk yield ($P = 0.09$), 3.5% FCM ($P = 0.05$), ECM ($P = 0.05$), milk fat yield ($P = 0.08$), milk protein yield ($P = 0.03$), and milk lactose yield ($P = 0.05$). **Linear (L) contrasts ($P < 0.05$) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM. Values shown in Figure 1 are also reported in Supplemental Table S3 (<https://doi.org/10.3168/jds.2019-16374>).

of de novo FA, increasing *cis*-9 C18:1 in FA treatments tended to decrease the yield of mixed milk FA (linear, $P = 0.06$).

We observed an interaction between FA treatments and production level for the yield of de novo milk FA ($P = 0.10$; Table 6) due to increasing dietary *cis*-9 C18:1 in FA treatments decreasing the yield of de novo FA in low-producing cows while increasing yield in high-producing cows (linear, $P < 0.05$; Figure 2A). Increasing dietary *cis*-9 C18:1 in FA treatments decreased the yield of mixed FA in low- and medium-producing cows but did not affect it in high-producing cows (quadratic interaction; $P = 0.10$; Figure 2B). Also, increasing dietary *cis*-9 C18:1 in FA treatments increased preformed FA yield in medium-producing (quadratic interaction; $P = 0.01$) and high-producing (linear; $P = 0.01$; Figure 2C) cows.

Blood Metabolites

Although FA treatments did not affect plasma non-esterified fatty acids concentration ($P = 0.48$; Table 7), increasing *cis*-9 C18:1 in FA treatments increased BHB (linear, $P = 0.05$) and tended to increase insulin (linear, $P = 0.08$) concentrations. We also observed an interaction between FA treatments and production level for plasma insulin ($P = 0.06$) due to increasing dietary *cis*-9 C18:1 in FA treatments linearly increasing insulin in low-producing cows ($P < 0.05$) while quadratically affecting insulin in high-producing cows ($P < 0.05$; Figure 3A).

Calculated Energy and Energy Partitioning

Increasing *cis*-9 C18:1 in FA treatments increased energy output in body reserves (quadratic, $P = 0.05$; Table 8) without changing milk energy output and energy for maintenance. We observed an interaction between FA treatments and production level for the intake of digestible energy, ME, and NE_L ($P < 0.10$) due to increasing dietary *cis*-9 C18:1 in FA treatments increasing these variables in high-producing cows but not in low- and medium-producing cows (linear, $P < 0.05$; Figure 3B–D). We also observed an interaction between FA treatments and production level for milk energy output ($P = 0.04$) and energy partitioned to milk ($P = 0.05$) due to increasing dietary *cis*-9 C18:1 in FA treatments reducing these variables in low-producing cows while increasing them in high-producing cows (linear, $P < 0.05$; Figure 3E–F).

DISCUSSION

Post-peak cows are usually in positive energy balance, and the goals are to optimize the yield of milk and milk components to maximize milk price and income. Typically, C16:0 and *cis*-9 C18:1 are the most abundant FA found in commercially available FA supplements fed to dairy cows. Also, these FA normally compose the majority of FA present in milk fat, ranging from 20 to 40 and from 20 to 30 g/100 g of FA for C16:0 and *cis*-9 C18:1, respectively (Jensen, 2002). Although we recently observed that altering the dietary ratio of

Table 6. Fatty acid (FA) concentration and yield by source of milk FA for cows fed treatment diets (n = 36)

Variable ¹	Treatment ²					P-value ³			Contrast ⁴		
	80:10	73:17	66:24	60:30	SEM	Trt	Production	Trt × production	Linear	Quadratic	Cubic
Summation by source, g/100 g of FA											
De novo	25.6	24.9	24.9	25.4	0.39	0.23	0.14	0.59	0.60	0.04	0.84
Mixed	40.9	39.6	39.1	37.9	0.38	<0.01	0.18	0.78	<0.01	0.87	0.21
Preformed	33.5	35.5	35.9	36.7	0.56	<0.01	0.93	0.54	<0.01	0.17	0.37
Summation by source, g/d											
De novo	414	410	412	418	16.1	0.96	0.29	0.10	0.81	0.64	0.98
Mixed	665	654	645	628	21.3	0.10	0.02	0.10	0.06	0.81	0.86
Preformed	540	576	591	596	14.5	<0.01	<0.01	0.01	<0.01	0.16	0.81

¹De novo FA originated from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originated from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual FA are reported in Supplemental Tables S1 and S2 (<https://doi.org/10.3168/jds.2019-16374>), respectively.

²80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

³P-values associated with treatment (Trt), production level, and interaction.

⁴Contrast refers to the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

Figure 2. Effects of varying the dietary ratio of palmitic and oleic acids on yield of (A) de novo milk fatty acids (FA), (B) mixed FA, and (C) preformed FA of dairy cows across different production levels (low = 45.2 ± 1.7 kg/d; medium = 53.0 ± 1.6 kg/d; high = 60.0 ± 1.9 kg/d). Treatments were as follows: 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between FA treatments and production level were detected for de novo ($P = 0.10$), mixed ($P = 0.10$), and preformed ($P = 0.01$) milk FA. **Linear (L) and quadratic (Q) contrasts ($P < 0.05$) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM. Values shown in Figure 2 are also reported in Supplemental Table S4 (<https://doi.org/10.3168/jds.2019-16374>).

C16:0 and *cis*-9 C18:1 may alter nutrient partitioning between the mammary gland and adipose tissue, only 1 combination of these FA was evaluated (de Souza et al., 2018). Therefore, our aim in the current study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in supplemental fat on performance of mid-lactation dairy cows. Additionally, cow milk production level was used as a blocking factor to investigate possible interactions between dietary treatments and metabolic demand. Although production level blocks were similar in DIM, they differed in average yields of milk and milk components. Evaluating potential interactions between dietary strategies and production level is important. This information is readily available to the dairy producer and can be easily used for grouping and feeding cows.

The effect of fat supplements on DMI is variable and usually depends on the profile of the FA supplement being fed (Allen, 2000; Rabiee et al., 2012). Harvatine and Allen (2006b) observed that DMI decreased linearly as the degree of unsaturation of supplemental fat increased. The more pronounced decrease in DMI for UFA is likely mediated in part by increased secretion of gut peptides related to satiety (Bradford et al., 2008). In contrast, in our study we did not observe changes in DMI as we increased *cis*-9 C18:1 in FA treatments, and we observed no interaction between FA treatments and production level. de Souza et al. (2018) reported that feeding an FA blend with 45% C16:0 and 35% *cis*-9 C18:1 decreased DMI compared with a nonfat control diet in the whole-cottonseed basal diet but not in the soyhull basal diets, which is likely related to the greater intake and duodenal flow of UFA on the whole-cottonseed basal diet. He et al. (2012) did not observe effects on DMI of varying levels of *cis*-9 C18:1 (from 0.98 to 3.32% of diet DM), but DMI was linearly reduced as *cis*-9, *cis*-12 C18:2 concentration increased in the diet (from 1.51 to 3.86% of diet DM). These results may indicate that the depression in feed intake caused by supplemental fat may be related to the level of supplemental fat, amount of UFA in the supplement and basal diet, potential interaction with other dietary components, and specific FA or degree of unsaturation. In our study, although increasing dietary *cis*-9 C18:1 in supplemental fat mainly increased *cis*-9 C18:1, *cis*-9, *cis*-12 C18:2 intake also increased. Further research is needed to determine whether higher amounts of UFA in general or a greater amount from a specific FA is related to depression in feed intake.

Additionally, we observed that increasing *cis*-9 C18:1 in FA treatments increased digestibilities of total, 16-carbon, and 18-carbon FA. Likewise, Harvatine and Allen (2006b) observed that total, 16-carbon, and 18-carbon FA digestibilities increased linearly as the

Table 7. Blood metabolites for cows fed the treatment diets (n = 36)

Variable	Treatment ¹					P-value ²			Contrast ³		
	80:10	73:17	66:24	60:30	SEM	Trt	Production	Trt × production	Linear	Quadratic	Cubic
NEFA, ⁴ mEq/L	0.10	0.11	0.11	0.11	0.005	0.48	0.26	0.20	0.51	0.23	0.46
BHB, mg/dL	7.76	8.43	8.81	9.35	0.31	0.10	0.01	0.88	0.05	0.21	0.42
Insulin, µg/L	0.58	0.62	0.63	0.65	0.03	0.10	0.15	0.06	0.08	0.72	0.78

¹80:10 = 1.5% of fatty acids (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²P-values associated with treatment, production level, and interaction.

³Contrast refers to the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

⁴NEFA = nonesterified fatty acid.

degree of unsaturation of supplemental fat increased. Usually, UFA have higher digestibility than SFA (Boerman et al., 2015), which may be due to the greater solubility of UFA facilitating transfer of FA to micelles (Freeman, 1969) and rapid uptake and re-esterification in enterocytes compared with SFA (Ockner et al., 1972). Recently, Rico et al. (2017) observed that 16-carbon FA digestibility was reduced when feeding a C16:0 supplement (85% C16:0) to a greater extent in a soyhulls basal diet compared with a whole-cottonseed basal diet. Although total flow of FA at the duodenum affects FA digestibility (Boerman et al., 2015), our findings support the hypothesis that the profile of FA entering the duodenum is a critical factor affecting FA digestibility. Furthermore, previous results have indicated that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0 (Boerman et al., 2015), and *cis*-9 C18:1 has been suggested as having amphiphilic properties (Moate et al., 2004). This is supported by Freeman (1969), who examined the amphiphilic properties of polar lipid solutes and reported that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. Additional research to understand the mechanisms by which *cis*-9 C18:1 may increase digestibility of other FA is required.

In our study, there was no effect of treatment on NDF digestibility. Similarly, de Souza et al. (2018) observed no differences in NDF digestibility when feeding an FA blend with a high content of C16:0 (80% C16:0) and an FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1), whereas both treatments increased NDF digestibility compared with a nonfat control diet and a diet supplemented with an FA blend of 40% C16:0 and 40% C18:0. Previous studies feeding C16:0 supplements indicated increases in NDF digestibility compared with a nonfat control diet (de Souza et al., 2017; Rico et al., 2017). In a recent meta-analysis, Weld and Armentano (2017) observed that Ca salts of palm FA and saturated fat containing a mixture of C16:0 and C18:0 did not affect NDF

digestibility. Piantoni et al. (2015) did not observe treatment × production level interactions for DM and OM digestibility when cows were fed a C18:0 supplement. Interestingly, we also did not observe interactions between FA treatment and production level for DM digestibility; however, digestible energy, ME, and NE_L intake increased with increasing dietary *cis*-9 C18:1 in FA treatments in high-producing cows but not in low- and medium-producing cows. Most studies in the literature predicted energy intake from diet ingredients rather than from nutrient digestibility determination, as we did in our current study. Therefore, reasons for inconsistent results regarding energy intake may include not only the duration of the supplementation period and the FA profile of supplemental fat but also different methods to calculate or predict energy intake.

To our knowledge, responses to altering the dietary ratio of FA have not been previously reported using cows at different levels of production. We observed a tendency for increasing *cis*-9 C18:1 in FA treatments increasing milk yield, which was driven by the effect of FA treatments on high-producing cows. In our study, higher-yielding cows responded more favorably to an FA blend containing a higher content of *cis*-9 C18:1 for yields of milk and milk components compared with lower-yielding cows. Previous research has shown that the production and metabolic responses to FA supplements can differ in cows at different levels of milk production (Harvatine and Allen, 2005; Warntjes et al., 2008). A previous study reported that C16:0 supplementation did not interact with level of milk production and, therefore, that cows with a wide range of milk production responded similarly to treatment (Piantoni et al., 2013). In contrast, DMI and milk production increased with C18:0 supplementation for higher-producing cows than for lower-producing cows (Piantoni et al., 2015). These results suggest that higher-producing cows may respond better to supplements containing 18-carbon FA. Although we increased dietary *cis*-9 C18:1, it is likely

Figure 3. Effects of varying the dietary ratio of palmitic and oleic acids on (A) plasma insulin, (B) digestible energy (DE) intake, (C) ME intake, (D) NE_L intake, (E) milk energy output, and (F) energy partitioned to milk of dairy cows across different production levels (low = 45.2 ± 1.7 kg/d; medium = 53.0 ± 1.6 kg/d; high = 60.0 ± 1.9 kg/d). Treatments were as follows: 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between FA treatments and production level were detected for plasma insulin ($P = 0.06$), DE intake ($P = 0.07$), ME intake ($P = 0.06$), NE_L intake ($P = 0.05$), milk energy output ($P = 0.04$), and energy partitioned to milk ($P = 0.05$). **Linear (L) and quadratic (Q) contrasts ($P < 0.05$) of the effects of *cis*-9 C18:1 inclusion in supplemental fat. Error bars represent SEM. Values shown in Figure 3 are also reported in Supplemental Table S5 (<https://doi.org/10.3168/jds.2019-16374>).

Table 8. Calculated energy and energy partitioning toward milk, body tissues, and maintenance for cows fed treatment diets (n = 36)

Variable	Treatment ¹					P-value ²			Contrast ³		
	80:10	73:17	66:24	60:30	SEM	Trt	Production	Trt × production	Linear	Quadratic	Cubic
Energy intake, Mcal/d											
GE ⁴	133	133	134	134	2.45	0.89	0.01	0.91	0.82	0.99	0.89
DE ⁵	90.2	91.2	91.1	92.3	1.47	0.65	0.01	0.07	0.24	0.97	0.66
ME ⁶	78.2	79.2	79.2	80.0	1.29	0.67	0.01	0.06	0.25	0.93	0.68
NE _L ⁷	48.6	50.0	50.4	50.4	0.98	0.40	0.07	0.05	0.13	0.42	0.83
Energy output, Mcal/d											
Milk ⁸	33.2	34.0	34.1	34.4	0.83	0.52	<0.01	0.04	0.10	0.67	0.76
Body reserves ⁹	4.60	5.10	5.40	5.60	0.44	0.08	0.33	0.48	0.05	0.01	0.18
Maintenance ¹⁰	11.0	10.9	10.9	11.0	0.12	0.11	0.07	0.68	0.97	0.17	0.77
Partitioning, % of energy intake											
Milk	68.3	68.0	67.7	68.2	1.51	0.24	0.03	0.01	0.12	0.27	0.39
Body reserves	9.5	10.2	10.7	11.1	0.91	0.82	0.12	0.40	0.11	0.86	0.51
Maintenance	22.6	21.8	21.6	21.8	1.82	0.28	0.16	0.47	0.10	0.53	0.39

¹80:10 = 1.5% of fatty acids (FA) supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 73:17 = 1.5% of FA supplement blend to provide ~73% C16:0 and 17% *cis*-9 C18:1; 66:24 = 1.5% of FA supplement blend to provide ~66% C16:0 and 24% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²P-values associated with treatment, production level, and interaction.

³Contrast refers to the linear, quadratic, and cubic effects of *cis*-9 C18:1 inclusion in supplemental fat.

⁴Gross energy intake (Mcal/d) = diet gross energy × feed intake.

⁵Digestible energy intake = gross energy intake (Mcal/d) × gross energy digestibility.

⁶ME intake was calculated from DE according to NRC (2001).

⁷NE_L intake was calculated from DE through ME according to NRC (2001).

⁸Milk NE_L (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)] (NRC, 2001).

⁹Body reserves (Mcal/d) = (2.88 + 1.036 × BCS) × ΔBW, where BCS was the average BCS for the study and ΔBW was BW change.

¹⁰NE_L maintenance (Mcal/d) = NE_L intake (Mcal/d) – milk NE_L (Mcal/d) – NE_L reserves (Mcal/d).

that this treatment increased rumen outflow of other 18-carbon FA, so it is unclear whether these results are associated with an overall effect of 18-carbon FA or a specific FA. Bionaz et al. (2013) indicated that FA can affect gene expression of several metabolic pathways. Gluconeogenesis in perfused liver of rats was stimulated by *cis*-9 C18:1 (Teufel et al., 1967), possibly indicating a role of this FA in mediating liver glucose metabolism. Conversely, White et al. (2011) examined the effect of different FA on gene expression of rat hepatoma cells transfected with specific bovine promoters and showed that *cis*-9 C18:1 did not affect the expression of gluconeogenic enzymes, whereas C18:0 decreased expression of pyruvate carboxylase, a key gluconeogenic enzyme. Although results from the latter experiment suggest that both C18:0 and *cis*-9 C18:1 would either not affect or decrease milk yield through a decrease in gluconeogenesis, which is contrary to our results, we cannot rule out a potential effect of these FA in the liver or other tissues.

We observed an interaction between FA treatments and production level for milk energy output and energy partitioning to milk. This was due to increasing *cis*-9 C18:1 in FA treatments decreasing milk fat yield and ECM in low-producing cows but increasing these in

high-producing cows. In cows averaging 51 kg/d at the start of the study, de Souza et al. (2018) observed that, compared with a nonfat control diet, ECM and milk energy output increased when feeding an FA blend with high content of C16:0 (80% C16:0) but not an FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1). Previous studies have reported that C16:0 supplementation increased ECM (Piantoni et al., 2013; de Souza et al., 2016, 2017), and this increase was consistent across a wide range of milk production levels (Piantoni et al., 2013; Rico et al., 2014b). Similar to our results, 3.5% FCM and ECM increased with C18:0 supplementation for higher-producing cows compared with lower-producing cows (Piantoni et al., 2015). In contrast, Rico et al. (2014a) did not observe treatment differences for ECM when evaluating the effects of a C16:0 supplement and a Ca salts of palm FA supplement in low-producing (average = 34 kg/d) and high-producing (average = 48 kg/d) cows. Although all the above-mentioned studies were conducted with cows after peak production, there was a wide range in DIM within and across studies, which suggests that other animal factors (e.g., pregnancy status, health status) may interact with nutrition strategies, thereby affecting animal responses. It is important to point out

that in our trial, the low-producing cows averaged 45 kg/d, so further research is needed to assess the effects of dietary ratio of FA in cows with a lower level of milk production. Based on our results and those from Piantoni et al. (2015), further studies are needed to determine whether high-producing cows may respond better to supplements containing 18-carbon FA per se or to a specific 18-carbon FA.

In low-producing cows, increasing *cis*-9 C18:1 in supplemental fat decreased milk fat yield due to a decrease in de novo and mixed milk FA without changes in preformed milk FA. In contrast, in high-producing cows, increasing *cis*-9 C18:1 in supplemental fat increased milk fat yield due to an increase in de novo and preformed milk FA. Hansen and Knudsen (1987) reported that C16:0 stimulated de novo FA synthesis and incorporation into triglycerides, whereas other FA (C18:0, C18:1, and C18:2) had no effect in dispersed goat mammary epithelial cells. In our study, the effect of FA treatments on milk FA profile was influenced by production level. Dorea and Armentano (2017) observed in a meta-analysis a negative relationship between dietary *cis*-9 C18:1 content and de novo milk FA yield. This substitution effect of preformed for de novo milk FA has been reported previously (He and Armentano, 2011; He et al., 2012), in which the reduction in yield of de novo milk FA was compensated for by an increase in the yield of preformed milk FA when fat supplements were fed. In contrast, Glasser et al. (2008) suggested that a positive relationship between de novo synthesized and long-chain FA can be expected in low-fat diets; however, when FA are supplemented to the diet, a simultaneous decrease in de novo FA and increase in long-chain FA occurs, corresponding to an inverse relationship between these 2 FA sources (Enjalbert et al., 1998; Glasser et al., 2008). Our results indicate an interdependence between de novo and preformed FA in high-producing cows driving milk fat yield, whereas in low-producing cows a substitution effect occurred. Similar to our results, Piantoni et al. (2015) also observed that C18:0 supplementation increased milk fat yield in higher-producing cows compared with lower-producing cows due to an increase in both de novo and preformed milk FA.

Regardless of production level, increasing *cis*-9 C18:1 in supplemental fat increased BW and BCS change and tended to increase energy partitioning to body reserves. Although increasing dietary *cis*-9 C18:1 did not cause milk fat depression because milk fat yield was unchanged, we observed a linear reduction in milk fat content and an increase in the concentration and yield of several *trans* FA in milk fat, suggesting a mild milk fat depression condition. Conceivably, increasing dietary *cis*-9 C18:1 provided a higher load of UFA in

the diet, which likely overcame normal rumen biohydrogenation capacity and changed biohydrogenation pathways. This is likely associated with repartitioning of energy toward body fat reserves. Recently, Urrutia and Harvatine (2017) observed reduced lipogenic capacity of adipose tissue explants without changes in gene expression of key lipogenic enzymes during 4 d of *trans*-10, *cis*-12 C18:2 infusion. In our study, we did not detect levels of *trans*-10, *cis*-12 C18:2 in milk fat of most of our samples, but it is important to consider that other FA produced as intermediates in rumen biohydrogenation have been shown to reduce milk fat (Bauman et al., 2011) and potentially may be involved with energy partitioning. Additionally, we observed that increasing dietary *cis*-9 C18:1 increased plasma insulin. Similarly, we observed higher plasma insulin levels in post-peak cows when feeding an FA blend containing 45% C16:0 and 35% *cis*-9 C18:1 compared with control and other FA-supplemented diets containing C16:0 and C18:0 (de Souza et al., 2018). Previous studies using the rat as an animal model have observed that free FA, including *cis*-9 C18:1, may stimulate insulin secretion from pancreatic β -cells (Itoh et al., 2003; Fujiwara et al., 2005). Insulin is an antilipolytic hormone, and elevated insulin concentrations may reduce lipolysis or increase lipogenesis in adipose tissue (Vernon, 2005). Furthermore, Yanting et al. (2018) reported that *cis*-9 C18:1 increased adipocyte number and size through enhancing adipogenic commitment and lipogenesis compared with SFA (C14:0, C16:0, and C18:0). Also, they reported that in mature adipocytes treated with FA, the lipid content in adipocytes increased with *cis*-9 C18:1 compared with C14:0, C16:0, C18:0, and *cis*-9, *cis*-12 C18:2 (Yanting et al., 2018). Therefore, the effect of *cis*-9 C18:1 on energy partitioning to body reserves could be linked to increased insulin concentrations, production of biohydrogenation intermediates, or both.

CONCLUSIONS

Regardless of production level, increasing *cis*-9 C18:1 in an FA supplement did not affect DMI but increased FA digestibility, BW change, and BCS change. Our results indicate that higher-producing dairy cows (averaging 60 kg/d) responded better to FA supplements containing more *cis*-9 C18:1, whereas lower-producing cows (averaging 45 kg/d) responded better to FA supplements containing more C16:0. Increasing *cis*-9 C18:1 did not affect milk yield and milk protein yield in low- and medium-producing cows but linearly increased these in high-producing cows. Increasing *cis*-9 C18:1 in an FA supplement linearly reduced FCM and ECM in low-producing cows but linearly increased these in high-producing cows.

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