J. Dairy Sci. 104:8673–8684
https://doi.org/10.3168/jds.2020-19913

Effect of palmitic acid-enriched supplements containing stearic or oleic acid on nutrient digestibility and milk production of low- and high-producing dairy cows

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ABSTRACT

We evaluated the effects of fatty acid (FA) supplement blends containing 60% palmitic acid (C16:0) and either 30% stearic acid (C18:0) or 30% oleic acid (*cis*-9 C18:1) on nutrient digestibility and milk production of low- and high-producing dairy cows. Twenty-four multiparous Holstein cows [118 ± 44 d in milk (DIM)] were divided into 2 blocks by milk production and then randomly assigned to treatment sequence in four 3 × 3 Latin squares within production level, balanced for carryover effects in three consecutive 21-d periods. Cows were blocked by milk yield and assigned to 1 of 2 groups (n = 12 per group): (a) low group (42.5 ± 3.54 kg/d; 147 ± 42 DIM) and (b) high group (55.8 ± 3.04 kg/d; 101 ± 34 DIM). Commercially available fat supplements were combined to provide treatments that consisted of the following: (1) control (CON; diet with no supplemental FA), (2) FA supplement blend containing 60% C16:0 and 30% C18:0 (PA+SA), and (3) FA supplement blend containing 60% C16:0 and 30% *cis*-9 C18:1 (PA+OA) The FA blends were fed at 1.5% of dry matter (DM) and replaced soyhulls from CON. Preplanned contrasts were (1) overall effect of FA treatments [CON vs. the average of the FA treatments (FAT); 1/2 (PA+SA + PA+OA)], and (2) effect of FA supplement (PA+SA vs. PA+OA). Regardless of production level, overall FAT reduced DMI compared with CON. Also, regardless of level of milk production, PA+OA increased total-tract FA digestibility compared with PA+SA. Treatment by production level interactions were observed for neutral detergent fiber (NDF) digestibility, total FA intake, and the yields of 3.5% fat-corrected milk (FCM), energy-corrected milk (ECM), and milk fat. In low-producing cows, FAT increased DM and NDF digestibility compared with CON. In high-producing cows PA+SA increased DM and NDF digestibility compared with PA+OA. In low-producing cows, PA+SA increased 3.5% FCM, ECM, and milk fat yield compared with PA+OA. However, in high-producing cows PA+OA tended to increase 3.5% FCM compared with PA+SA. In conclusion, low-producing cows responded better to a FA blend containing 60% C16:0 and 30% C18:0, whereas high-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1. However, further research is required to validate our observations that higher-yielding cows have improved production responses when supplemented with *cis*-9 C18:1 compared with C18:0.

Key words: stearic acid, oleic acid, production level, dairy cow

INTRODUCTION

Fat supplements are commonly added to dairy cow diets to help increase the yields of milk and milk fat (Rabiee et al., 2012). Research is progressing from feeding traditional animal and plant fats to feeding individual fatty acids (**FA**) and blends of FA, which extends beyond their energy contribution to include potentially structural, metabolic, and physiological effects (Palmquist and Jenkins, 2017). The most abundant FA in commercially available fat supplements fed to dairy cows are palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids. These 3 FA are also the predominant FA found in milk fat and adipose tissue (Palmquist, 2006). Attention has been given to these FA to understand their effects on nutrient and FA digestibility, milk production, and energy partitioning, and research suggests that dairy cows have different metabolic and production responses when fed combinations of these FA. For example, de Souza et al. (2018) observed that feeding a FA blend containing 80% C16:0 increased milk energy output, whereas a FA blend of 45% C16:0 and 35% *cis*-9 C18:1 increased energy storage as BW, and a combination of 40% C16:0 and 40% C18:0 decreased nutrient digestibility and did not increase energy intake.

Received November 13, 2020.
Accepted March 22, 2021.
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Previous research has also observed that cows at different levels of milk production can have different responses to dietary treatments. Supplementation with C16:0 increased yields of milk, milk fat, and ECM across a wide milk production compared with no FA supplementation (Piantoni et al., 2013) or compared with C18:0 supplementation (Rico et al., 2014b). However, Piantoni et al. (2013) observed in one treatment period that C18:0 supplementation increased yield of milk and milk fat in high-producing cows but not in low-producing cows. Recently, de Souza et al. (2019) varied the ratio of supplemental C16:0 to *cis*-9 C18:1 and observed that increasing the level of *cis*-9 C18:1 in a FA supplement blend increased ECM yield in high-producing cows but reduced ECM yield in low-producing cows. Western et al. (2020b) observed similar results across a wide range of milk production, with a supplement blend of 60% C16:0 + 30% *cis*-9 C18:1 increasing ECM yield in high-producing cows compared with a blend of 80% C16:0 + 10% *cis*-9 C18:1.

In our recent research, higher-producing dairy cows increased production when supplemented higher levels of *cis*-9 C18:1, with the *cis*-9 C18:1 being supplemented via a calcium salt of palm FA (de Souza et al., 2019; Western et al., 2020b). Calcium salts of palm FA are a common type of fat supplement developed to minimize the negative effects of UFA on rumen microbes, that have been reported to be effective in increasing milk production and milk fat percentage (Rabiee et al., 2012; Palmquist and Jenkins, 2017). Rumen bacteria convert UFA to SFA via biohydrogenation (**BH**) by the addition of hydrogen, with the main end product being C18:0 (Palmquist et al., 2005; Jenkins and McGuire, 2006). Calcium salts can dissociate in the rumen (Chalupa et al., 1986; Sukhija and Palmquist, 1990) and release UFA, allowing them to undergo BH (Hobson and Stewart, 1997). Because *cis*-9 C18:1 is BH to 18:0, we wondered whether the effects we have recently observed with *cis*-9 C18:1 were due to C18 FA in general or specifically due to *cis*-9 C18:1. Therefore, the objective of our study was to determine the effects of C16:0-enriched supplements containing C18:0 or *cis*-9 C18:1 on nutrient digestibility and milk production in low- and high-producing cows. We hypothesized that, compared with C18:0, *cis*-9 C18:1 would increase nutrient and FA digestibility, yield of milk components, and body reserve gain of high-producing cows.

MATERIALS AND METHODS

Design and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University, East Lansing. Twenty-four mid-lactation, multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were divided into 2 blocks by milk production and then randomly assigned to treatment sequence in four 3 × 3 Latin squares within production level, balanced for carryover effects in three 21-d periods. Animals were blocked by production level into 2 groups (n = 12 per group): (a) low group (42.5 ± 3.54 kg/d; 147 ± 42 DIM) and (b) high group (55.8 ± 3.04 kg/d; 101 ± 34 DIM). Baseline values for both production groups are provided in Table 1. All animals received a common diet with no FA supplementation during a 7-d preliminary period to obtain baseline values

Table 1. Baseline data for low- and high-producing cows in the study[1]

	Production level[2]	
Variable	Low	High
DIM	147 ± 42	101 ± 34
Milk yield, kg/d	42.5 ± 3.54	55.8 ± 3.04
Fat	1.52 ± 0.30	1.74 ± 0.17
Protein	1.36 ± 0.15	1.64 ± 0.11
Lactose	2.09 ± 0.26	2.82 ± 0.21
Milk composition, %		
Fat	3.56 ± 0.57	3.13 ± 0.37
Protein	3.21 ± 0.34	2.94 ± 0.22
Lactose	4.90 ± 0.33	5.05 ± 0.11
BW, kg	694 ± 81	713 ± 39
BCS	3.39 ± 0.39	3.16 ± 0.14

[1]Obtained during the preliminary period when cows were fed a common diet (mean ± SD).
[2]Low production level, n = 12; high production level, n = 12.

Within each production level group, a 3 × 3 balanced Latin square design was used to assign treatments. Treatments consisted of (1) control (**CON**; diet with no supplemental FA), (2) FA supplement blend containing 60% C16:0 and 30% C18:0 (**PA+SA**), and (3) FA supplement blend containing 60% C16:0 and 30% *cis*-9 C18:1 (**PA+OA**). Both FA supplement blends were fed at 1.5% FA (DM basis) of the diet and replaced soyhulls from the control diet. We used different combinations of 3 commercially available products that differed in FA profile (Table 2); these commercial products were blended to achieve our desired ratios of C16:0, C18:0, and *cis*-9 C18:1 in the FA supplement blends (Table 2). The ingredient and nutrient compositions of the diets fed as TMR were formulated to meet the requirements of the average cow in the study (NRC, 2001; Table 3). The DM concentrations of forages were determined twice weekly and diets adjusted when necessary. A base diet mix (containing forages, dry ground corn, high-moisture corn, soybean meal, and mineral mix) was mixed in a wagon daily. Then, soyhulls, FA supplement blends, and base diet were mixed in the

Table 2. Proportion of each commercial fatty acid (FA) supplement used in the FA blends and FA profile of each blend[1]

Item	FA treatment	
	PA+SA	PA+OA
FA supplement in treatment blends, %		
C16:0-enriched FA supplement[2]	49.5	27.8
C18:0-enriched FA supplement[3]	50.0	1.00
Calcium salt of palm FA supplement[4]	0.50	71.2
FA profile of each FA blend, g/100 g of FA		
C14:0	2.08	0.87
C16:0	59.3	59.3
C18:0	27.3	3.39
cis-9 C18:1	6.88	28.6
cis-9,*cis*-12 C18:2	1.29	6.41
cis-9,*cis*-12,*cis*-15 C18:3	0.02	0.17

[1]Average (n = 3) based on samples taken during the last 5 d of experimental periods. PA+SA = 60% C16:0 and 30% C18:0; PA+OA = 60% C16:0 and 30% *cis*-9 C18:1.

[2]Bergafat F-100 (Berg and Schmidt America LLC). FA composition, g/100 g of total FA: C14:0 = 0.41, C16:0 = 90.8, C18:0 = 0.27, *cis*-9 C18:1 = 6.30, *cis*-9,*cis*-12 C18:2 = 1.60, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.01.

[3]Energy Booster 100 (Milk Specialties Global). FA composition, g/100 g of total FA: C14:0 = 3.76, C16:0 = 28.4, C18:0 = 54.3, *cis*-9 C18:1 = 7.07, *cis*-9,*cis*-12 C18:2 = 0.90, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.03.

[4]Megalac (Church and Dwight Co. Inc.). FA composition, g/100 g of total FA: C14:0 = 1.02, C16:0 = 47.6, C18:0 = 3.93, *cis*-9 C18:1 = 37.3, *cis*-9,*cis*-12 C18:2 = 8.28, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.23.

Table 3. Ingredient and nutrient composition of treatment diets

Item	Treatment[1]		
	CON	PA+SA	PA+OA
Ingredient, % DM			
Corn silage	38.2	38.2	38.2
Alfalfa silage	21.1	21.1	21.1
Wheat straw	0.81	0.81	0.81
Ground corn	16.9	16.9	16.9
High-moisture corn	12.3	12.3	12.3
Soybean meal	11.3	11.3	11.3
Whole cottonseed	4.77	4.77	4.77
Protein supplement[2]	0.97	0.97	0.97
Soyhulls	7.09	5.61	5.46
PA+OA FA blend[3]	—	—	1.55
PA+SA FA blend[4]	—	1.45	—
Vitamin and mineral mix[5]	3.58	3.58	3.58
Nutrient composition, % DM			
NDF	29.8	28.9	28.8
Forage NDF	21.5	21.5	21.5
CP	15.7	15.5	15.5
Starch	31.9	32.0	32.0
FA	2.53	3.89	4.02
C16:0	0.45	1.29	1.36
C18:0	0.07	0.45	0.12
cis-9 C18:1	0.44	0.55	0.89
cis-9,*cis*-12 C18:2	1.31	1.35	1.43
cis-9,*cis*-12,*cis*-15 C18:3	0.19	0.18	0.18

[1]CON = no fatty acid (FA) supplement; PA+SA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:0; PA+OA = 1.5% of FA supplement blend to provide approximately 60% of C16:0 + 30% of C18:1 *cis*-9.

[2]Spectrum Agriblue (Perdue Agribusiness). The supplement contained (% DM) 89.5 CP, 55.6 total EAA, 11.0 leucine, 8.0 valine, 7.8 lysine, 6.2 phenylalanine, 5.4 histidine, 5.2 methionine.

[3]Blend of Bergafat 100 (Berg and Schmidt America LLC), Energy Booster (Milk Specialties Global), and Megalac (Church and Dwight Co. Inc.) to reach a 60% C16:0 and 30% C18:0 FA profile.

[4]Blend of Bergafat 100, Energy Booster, and Megalac to reach a 60% C16:0 and 30% *cis*-9 C18:1 FA profile.

[5]Vitamin and mineral mix contained 34.1% dry ground shelled corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos (The Mosaic Co.), 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.

mixer wagon for each experimental diet. Cows were fed 115% expected intake at 1000 h daily. Feed access was blocked from 0800 to 1000 h for orts collection and offering of new feed. Cows were housed in individual tiestalls throughout the experiment, with water available ab libitum in each stall, which were bedded with sawdust and cleaned twice daily.

Data and Sample Collection

Samples and production data were collected during the last 5 d of each treatment period (d 17–21). Samples of all diet ingredients (0.5 kg) and orts (12.5%) were collected daily and composited by cow per 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) and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at −20°C until analyzed for FA composition. Fecal samples were taken every 15 h, resulting in 8 samples/cow per period and stored in a sealed plastic cup at −20°C. Fecal samples were later dried and composited by cow per period for analysis. Blood was also taken every 15 h and stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Plasma was transferred into microcentrifuge tubes and stored at −20°C until composited by cow per period. Body weights were measured 3 times per week, and 3 trained investigators determined BCS on a 5-point scale in increments of 0.25 on the last day of each period (Wildman et al., 1982).

Sample Analysis

Dietary 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 in a Wiley mill (1-mm screen; Arthur H. Thomas). Samples of feed ingredients, orts, and feces were analyzed for

NDF, indigestible NDF, starch, CP, and FA according to Boerman et al. (2017). Indigestible NDF was estimated as NDF after 240-h in vitro fermentation (Goering and Van Soest, 1970) and was used as an internal marker to estimate fecal output, to determine apparent total-tract digestibility of nutrients (Cochran et al., 1986). Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Dairy Herd Improvement Association (Central Star DHI, Grand Ledge, 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. Fat-corrected milk was calculated as 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]. Energy-corrected milk was calculated as ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)]. Milk samples used for analysis of FA composition were composited based on milk fat yield (d 17–21 per period). Milk lipids were extracted and FAME prepared and analyzed by gas chromatography, as described previously (Lock et al., 2013). Yields of individual FA (g/d) in milk fat 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 content and other milk lipid classes (Piantoni et al., 2013).

Statistical Analysis

All data were analyzed using the GLIMMIX model procedure of SAS (version 9.4, SAS Institute Inc.). Data were analyzed using the following model:

$$Y_{ijkl} = \mu + C(G)_{i(j)} + G_j + P_k + T_l + G_j \times T_l + e_{ijkl},$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, $C(G)_{i(j)}$ = random effect of cow nested in production group (i = 1–12), G_j = fixed effect of production level (j = 2), P_k = fixed effect of period (k = 1–3), T_l = fixed effect of treatment (l = 1–3), $G_j \times T_l$ = the interaction of production level and treatment, and e_{ijkl} = residual error. The interaction of period and treatment and the 3-way interaction of treatment, production level, and period were all tested, but results were not significant and were removed from the model ($P > 0.20$). Normality of the residuals was checked with the normal probability, 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 at $P \leq 0.10$, and interactions were declared significant at $P \leq 0.10$ and tendencies at $P \leq 0.15$. Denominator degrees of freedom were specified using the Kenward-Roger option and ranged from 40.4 to 42 for the fixed effects of treatment, and the interaction of production level and treatment and ranged from 21.9 to 22 for the fixed effect of production level. Two orthogonal contrasts were evaluated: (1) the overall effect of FA supplements {CON vs. the average of the FA treatments (**FAT**) [1/2 (PA+SA + PA+OA)]}, and (2) the effect of the PA+SA treatment versus the PA+OA treatment (PA+SA vs. PA+OA). These contrasts were used to test the main effect of FA treatments and also to test the effect of FA treatments within production level.

RESULTS

Nutrient Intake and Total-Tract Digestibility

Overall, compared with CON, FAT decreased DM and NDF intake and increased DM and NDF digestibility (all $P < 0.05$; Table 4). The FAT increased intakes of 16-carbon, 18-carbon, and total FA (all $P < 0.05$) compared with CON. Moreover, FAT increased 16-carbon digestibility ($P < 0.05$), did not affect 18-carbon digestibility ($P = 0.83$), and decreased total FA digestibility ($P < 0.05$) compared with CON. The FAT also increased absorbed 16-carbon, 18-carbon, and total FA (all $P < 0.05$) compared with CON.

Compared with PA+SA, PA+OA decreased NDF ($P < 0.04$) but did not affect DM intake ($P = 0.13$). The PA+OA treatment decreased DM digestibility ($P < 0.05$) and tended to decrease NDF digestibility ($P < 0.10$) compared with PA+SA. The PA+OA treatment increased intakes of 16-carbon, 18-carbon, and total FA (all $P < 0.05$) and increased 16-carbon, 18-carbon, and total FA digestibility (all $P < 0.05$) compared with PA+SA. Additionally, PA+OA increased absorbed 16-carbon, 18-carbon, and total FA (all $P < 0.05$) compared with PA+SA.

We observed interactions between FA treatments and production level for DM and NDF digestibility. In both low- and high-producing cows, FAT increased DM and NDF digestibility compared with CON (all $P < 0.10$; Figure 1), but the magnitude of difference was greatest in high-producing cows. In low-producing cows no difference in DM digestibility was detectable between PA+OA and PA+SA ($P = 0.79$), whereas in high-producing cows the PA+SA treatment increased DM digestibility ($P < 0.05$) compared with PA+OA. Similarly, in low-producing cows we detected no difference for NDF digestibility between PA+OA and PA+SA, whereas in high-producing cows PA+SA increased NDF digestibility ($P < 0.05$) compared with PA+OA.

Production Responses

Overall, FAT increased the yields of milk, 3.5% FCM, ECM, milk fat, milk lactose, and feed efficiency (ECM/DMI; all $P < 0.05$; Table 5), and decreased milk protein concentration and BW change ($P < 0.05$) compared with CON. The FAT decreased milk protein concentration ($P < 0.05$) due to the increase in milk and milk lactose yield, whereas milk protein yield remained constant. We detected no difference between CON and FAT for milk protein yield, milk fat and lactose concentrations, BW, BCS, or BCS change (all $P > 0.15$).

We detected no differences between PA+OA and PA+SA for the yields of milk, 3.5% FCM, ECM, milk fat, milk protein, and milk lactose, and ECM/DMI, BW, BW change, BCS, and BCS change (all $P > 0.20$; Table 5). The PA+SA treatment increased milk protein concentration ($P < 0.05$) compared with PA+OA.

We observed interactions between FA treatments and production level for many production responses. No difference between CON and FAT was detectable in low-producing cows for yields of milk, 3.5% FCM, ECM, milk fat, milk protein, and milk lactose (all $P > 0.33$; Figure 2), whereas in high-producing cows FAT increased yields of milk, 3.5% FCM, ECM, milk fat, milk lactose, and ECM/DMI (all $P < 0.05$) compared with CON. In both low- and high-producing cows, FAT increased ECM/DMI (both $P < 0.05$), but the magnitude of difference was greatest for high-producing cows. We found no difference between PA+OA and PA+SA for milk yield in both low- ($P = 0.27$) and high-producing cows ($P = 0.15$). The PA+SA treatment increased yields of 3.5% FCM, ECM, milk fat, and milk protein in low-producing cows (all $P < 0.05$), whereas the PA+OA treatment increased ECM/DMI (both $P < 0.05$) and tended to increase the yield of 3.5% FCM ($P = 0.08$) in high-producing cows compared with PA+SA. No difference between PA+OA and PA+SA was observed for yields of ECM ($P = 0.14$), milk fat ($P = 0.11$), milk protein ($P = 0.59$), or milk lactose ($P = 0.14$) in high-producing cows (all $P > 0.10$; Supplemental Table S2, https://dx.doi.org/10.5281/zenodo.4701271).

Milk FA Content and Yields

Milk FA are derived from 2 sources: de novo (<16 carbon FA) synthesis in the mammary gland and preformed (>16 carbon FA) originating from extraction from plasma. Mixed-source 16-carbon FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. Individual yields and concentrations of milk FA can be found in Supplemental Tables S3 and S4 (https://dx.doi.org/10.5281/zenodo.4701271), respectively. The FAT de-

Table 4. Nutrient intake and digestibility for cows fed treatment diets (n = 24)[1]

	Treatment[2]				*P*-value[4]			Contrasts[5]	
Variable	CON	PA+SA	PA+OA	SEM[3]	Trt	Prod	Trt × Prod	CON vs. FAT	PA+SA vs. PA+OA
Intake, kg/d									
DM	33.2	32.8	32.4	0.54	0.05	<0.01	0.77	0.02	0.13
NDF	9.90	9.34	9.20	0.15	<0.001	<0.001	0.84	<0.001	0.04
Intake, g/d									
16-carbon	117	347	371	5.51	<0.001	<0.001	0.01	<0.001	<0.001
18-carbon	549	692	722	11.3	<0.001	<0.001	0.22	<0.001	<0.01
Total FA	784	1,153	1,183	18.3	<0.001	<0.001	0.09	<0.001	0.02
Digestibility, %									
DM	66.1	68.8	68.2	0.26	<0.001	0.92	0.13	<0.001	0.09
NDF	41.6	44.8	43.4	0.43	<0.001	0.91	0.10	<0.01	0.05
16-carbon	66.5	58.4	69.0	1.32	<0.001	0.11	0.52	<0.001	<0.001
18-carbon	70.0	66.2	76.0	1.71	<0.001	0.14	0.79	0.83	<0.001
Total FA	72.3	65.7	74.4	1.44	<0.001	0.14	0.88	<0.001	<0.001
Absorbed, g/d									
16-carbon	77.3	203	255	4.27	<0.001	0.14	0.18	<0.001	<0.001
18-carbon	383	457	548	11.6	<0.001	0.33	0.59	<0.001	<0.001
Total FA	565	757	879	16.6	<0.001	0.18	0.50	<0.001	<0.001

[1]Experimental diets fed to 24 cows (12 low-producing cows; 12 high-producing cows) in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

[2]Treatments were CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1.

[3]Greatest SEM related to main effect of treatment.

[4]*P*-values refer to the ANOVA results for the fixed effects of treatment (Trt) and production level (Prod), and the interaction of Trt × Prod.

[5]CON vs. FAT and PA+SA vs. PA+OA contrasts tested the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1, respectively.

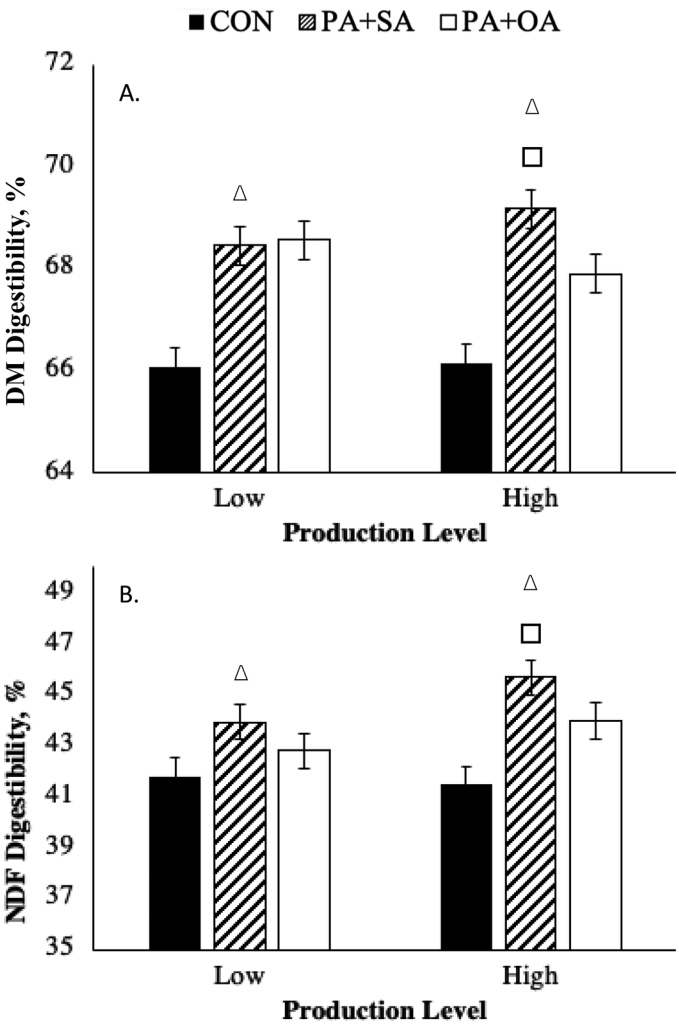

Figure 1. Effects of treatments on (A) DM digestibility and (B) NDF digestibility in low-producing (42.5 ± 3.54 kg/d) and high-producing (55.8 ± 3.04 kg/d) dairy cows. Treatments were as follows: CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1. Tendency for interactions between treatments and production level were detected for DM digestibility ($P = 0.13$) and NDF digestibility ($P = 0.10$). Error bars represent SEM used for each individual treatment. CON vs. FAT (Δ) and PA+SA vs. PA+OA (□) contrasts ($P < 0.10$) were to test the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1 within production level, respectively. See Supplemental Table S1 (https://dx.doi.org/10.5281/zenodo.4701271).

creased de novo milk FA yield and increased yields of mixed and preformed milk FA (all $P < 0.05$; Table 6) compared with CON. Similarly, FAT decreased de novo milk FA content and increased mixed and preformed milk FA contents (all $P < 0.05$) compared with CON.

The PA+OA treatment decreased de novo and mixed milk FA yields but increased preformed milk FA yield (all $P < 0.05$) compared with PA+SA. Also, PA+OA decreased de novo and mixed milk FA content but increased preformed milk FA content (all $P < 0.05$) compared with PA+SA.

We observed interactions between FA treatments and production level for milk FA yields and concentrations. In both low- and high-producing cows, FAT decreased de novo milk FA yield (both $P < 0.05$; Figure 3 and Supplemental Table S5, https://dx.doi.org/10.5281/zenodo.4701271) compared with CON, but the magnitude of decrease was greatest in low-producing cows. In both low- and high-producing cows, FAT increased mixed and preformed milk FA yields (both $P < 0.05$) compared with CON, but the magnitude of increase was greatest for high-producing cows. The PA+OA treatment decreased de novo and mixed milk FA yield in low-producing cows ($P < 0.05$), but in high-producing cows no difference was detectable between PA+OA and PA+SA for either variable ($P = 0.62$ and $P = 0.56$, respectively). We found no difference between PA+OA and PA+SA in low-producing cows for preformed milk FA yield ($P = 0.74$), but PA+OA increased preformed milk FA yield ($P < 0.01$) in high-producing cows.

Plasma Insulin

We did not observe any effect of FA treatments or FA treatment and production-level interactions for plasma insulin ($P > 0.22$; Supplemental Table S6, https://dx.doi.org/10.5281/zenodo.4701271).

DISCUSSION

Most commercially available fat supplements fed to dairy cows contain different proportions of C16:0, C18:0, and *cis*-9 C18:1 FA, and research highlights different effects of these FA on nutrient digestibility and production of dairy cows. Recent research suggests that high-producing cows may benefit from increased supplementation of *cis*-9 C18:1 (de Souza et al., 2019; Western et al., 2020b); however, these studies were not able to determine whether responses were due to dietary *cis*-9 C18:1 or 18-carbon FA. Therefore, in our current study, we used production level as a blocking factor to evaluate whether cows with differing levels of milk yield would respond differently to C16:0-enriched supplements containing either C18:0 or *cis*-9 C18:1. We designed the FA treatments to contain 60% C16:0 and either 30% C18:0 or 30% *cis*-9 C18:1 to evaluate whether production responses are due to specific effects of an individual FA or 18-carbon FA overall. We hypothesized that supplemental *cis*-9 C18:1 would increase nutrient digestibility, yield of milk components, and body reserve gain compared with a treatment containing C18:0 in high-producing cows.

Table 5. Milk yield, milk components, BW, and BCS for cows fed treatment diets (n = 24)[1]

	Treatment[2]				*P*-value[4]			Contrasts[5]	
Variable	CON	PA+SA	PA+OA	SEM[3]	Trt	Prod	Trt × Prod	CON vs. FAT	PA+SA vs. PA+OA
Yield, kg/d									
Milk	44.7	45.8	46.0	0.97	0.06	<0.01	0.12	0.02	0.81
3.5% FCM[6]	46.4	47.9	47.8	1.00	0.02	<0.01	0.01	<0.01	0.82
ECM[7]	46.9	48.2	47.8	0.94	0.09	<0.01	0.02	0.03	0.59
Fat	1.67	1.73	1.72	0.04	0.02	<0.01	<0.01	<0.01	0.56
Protein	1.50	1.51	1.48	0.03	0.43	<0.01	0.12	0.86	0.20
Lactose	2.15	2.20	2.21	0.06	0.15	<0.01	0.11	0.06	0.65
Milk composition, %									
Fat	3.78	3.86	3.80	0.09	0.37	<0.01	0.24	0.49	0.22
Protein	3.41	3.35	3.27	0.05	<0.01	<0.01	0.46	<0.01	<0.01
Lactose	4.79	4.78	4.78	0.07	0.52	0.10	0.33	0.27	0.82
ECM/DMI	1.41	1.46	1.47	0.03	<0.01	<0.01	0.02	<0.01	0.49
BW, kg	727	727	726	14.2	0.82	0.48	0.46	0.66	0.65
BW change, kg/d	0.77	0.48	0.22	0.13	0.02	0.56	0.91	0.01	0.17
BCS	3.33	3.35	3.32	0.07	0.67	0.03	0.81	0.83	0.38
BCS change	0.06	0.07	0.04	0.03	0.82	0.18	0.76	0.82	0.56

[1]Experimental diets fed to 24 cows (12 low-producing cows; 12 high-producing cows) in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

[2]Treatments were CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1.

[3]Greatest SEM related to main effect of treatment.

[4]*P*-values refer to the ANOVA results for the fixed effects of treatment (Trt) and production level (Prod), and the interaction of Trt × Prod.

[5]CON vs. FAT and PA+SA vs. PA+OA contrasts tested the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1, respectively.

[6]Fat-corrected milk: 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

[7]Energy-corrected milk: ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)]. This equation corrects milk to a 0.68 Mcal/kg energy basis.

C16:0 has consistently been shown to increase yields of milk and milk fat across a wide range of production levels (Piantoni et al., 2013; Rico et al., 2014a), whereas supplementation of C18:0 has had differing effects across production levels (Piantoni et al., 2015; Boerman et al., 2017). Studies observing effects of *cis*-9 C18:1 supplementation and differing ratios of C16:0, C18:0, and *cis*-9 C18:1 across production groups are scarce. Both de Souza et al. (2019) and Western et al. (2020b) observed that a ratio of 60% C16:0 + 30% *cis*-9 C18:1 had positive responses on production variables in higher-producing cows. Although we only observed an increase in the yield of 3.5% FCM in higher-producing cows for the PA+OA treatment, compared with the PA+SA treatment, other variables such as yields of milk, milk fat, and ECM (P = 0.15, 0.11, and 0.14, respectively) approached significance, indicating that additional work is required to further determine differences between supplemental C18:0 and *cis*-9 C18:1 in high-producing cows. Our FA treatments allowed for a direct evaluation of C18:0 versus *cis*-9 C18:1, and between our current study and previous research, these results indicate that higher-producing cows increase production due to *cis*-9 C18:1 and not 18-carbon FA or C18:0 per se. The tendencies observed in our study indicate that follow-up research should focus on the effects of a ratio of 60% C16:0 + 30% *cis*-9 C18:1 in high-producing dairy cows (>55 kg of milk/day) and the metabolic pathways that can explain the results observed within this production level.

At the start of our study, cows in our low-production group averaged 42.5 kg/d, and we observed that the PA+SA treatment increased yields of 3.5% FCM, ECM, and milk fat compared with our PA+OA treatment. Piantoni et al. (2015) observed that C18:0 supplementation in cows producing an average of 46.1 kg/d increased milk yield, milk fat yield, and 3.5% FCM compared with no fat supplementation. Supplementation of FA treatments containing ≥80% C16:0 and a blend of 80% C16:0 + 10% *cis*-9 C18:1 have been shown to increase yields of 3.5% FCM and ECM in lower-producing cows (Rico et al., 2014a; de Souza et al., 2019; Western et al., 2020b). For cows averaging 46.5 kg/d of milk yield, a FA blend of 80% C16:0 + 10% *cis*-9 C18:1 tended to increase 3.5% FCM and ECM compared with treatments containing 40% C16:0 + 40% C18:0 and a blend of 45% C16:0 + 35% *cis*-9 C18:1 (de Souza et al., 2018). Similarly, Western et al. (2020a) observed a FA treatment consisting of 80% C16:0 + 10% *cis*-9 C18:1 to increase yields of 3.5% FCM and ECM compared

with a FA treatment of 30% C16:0 + 50% C18:0 but did not evaluate production-level differences. Considering previous research, potential exists for a treatment higher in C16:0 to increase the yields milk components more compared with a treatment of C16:0 + C18:0 for low-producing cows. Future research should evaluate different ratios of C16:0 and C18:0 to assess whether these 2 FA have a synergistic effect in lower-producing cows.

One way to deliver *cis*-9 C18:1 is to use a fat supplement, such as a calcium salt. These salts are not completely rumen-inert, as there is some degree of dissociation of the calcium salt in the rumen (Chalupa et al., 1986), but this dissociation does not surpass ≥50% of the calcium salt at normal rumen pH levels (Sukhija and Palmquist, 1990). Due to this, we acknowledge and expect that some of the *cis*-9 C18:1 in the calcium salt used in our study will undergo rumen BH to C18:0

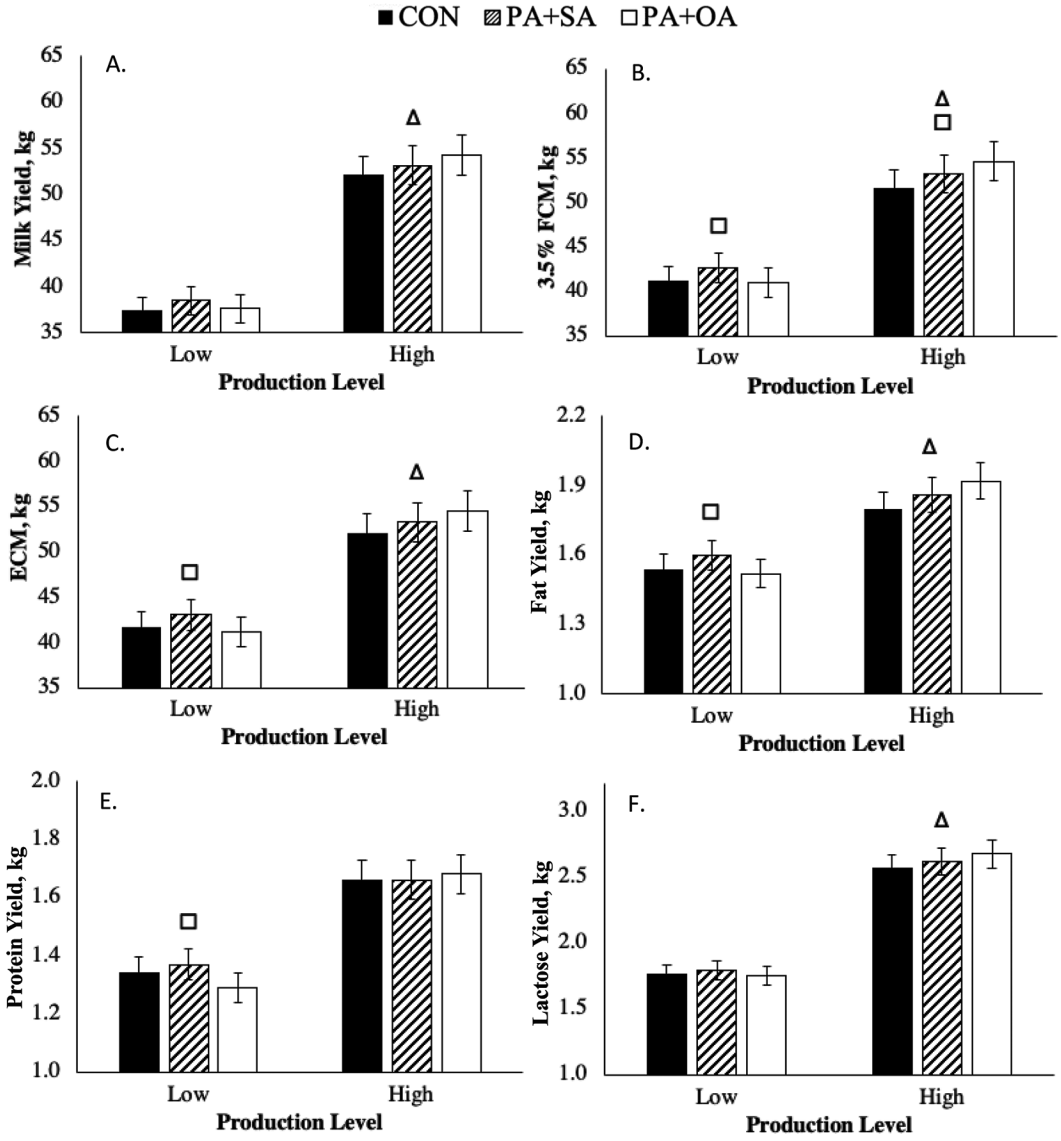

Figure 2. Effects of treatments on (A) milk yield, (B) 3.5% FCM, (C) ECM, (D) milk fat yield, (E) milk protein yield, and (F) milk lactose yield in low-producing (42.5 ± 3.54 kg/d) and high-producing (55.8 ± 3.04 kg/d) dairy cows. Treatments were as follows: CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between treatments and production level were detected for 3.5% FCM ($P = 0.01$), ECM ($P = 0.02$), and milk fat yield ($P < 0.01$). Tendency for interactions between treatments and production level were detected for milk yield ($P = 0.12$), protein yield ($P = 0.11$), and lactose yield ($P = 0.11$). Error bars represent SEM used for each individual treatment. CON vs. FAT (Δ) and PA+SA vs. PA+OA (□) contrasts ($P < 0.10$) were to test the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1 within production level, respectively. See Supplemental Table S2 (https://dx.doi.org/10.5281/zenodo.4701271).

(Carriquiry et al., 2008). To approximate how much *cis*-9 C18:1 was leaving the rumen, using calculations for our DMI and FA inclusion rate, we can approximate 100 g of *cis*-9 C18:1 in the calcium salt entering the rumen, and utilizing a 62% rumen loss for *cis*-9 C18:1 in a protected fat supplement (Jenkins and Bridges, 2007), we can estimate that ~40 g/d of *cis*-9 C18:1 was available in the small intestine for the cows in our study. If we use a range of 40 to 80% rumen loss, we can estimate that a range of ~20 to 60 g/d of *cis*-9 C18:1 will leave the rumen. Based on these estimates, we are increasing the supply of *cis*-9 C18:1 to the small intestine, which may have effects on FA digestibility and nutrient partitioning.

We observed that compared with PA+SA, the PA+OA treatment decreased de novo FA yield and increased preformed FA yield in high-producing cows, which is similar to results observed by de Souza et al. (2018). In contrast, de Souza et al. (2019) observed that high-producing cows increased milk fat yield due to increases in both de novo FA and preformed FA with a FA blend of 60% C16:0 + 30% *cis*-9 C18:1, and Piantoni et al. (2015) found similar results with C18:0 supplementation. In the high-producing cows, these results could be attributed to a substitution effect, where a decrease in the yield of de novo milk FA, which are compensated for by an increase in preformed milk FA, has often been observed in diets with added fat supplements (Glasser et al., 2008; He and Armentano, 2011; He et al., 2012). Similarly, a recent meta-analysis reported a negative relationship between dietary *cis*-9 C18:1 and de novo milk FA yield, and observed compensation of preformed milk FA due to the reduction in de novo milk FA yield (Dorea and Armentano, 2017). The PA+SA treatment reduced de novo milk FA yield and increased mixed milk FA yield in low-producing cows, which can explain the increase in milk fat yield. Similar to our low-producing cows, de Souza et al. (2019) observed reduction in de novo and mixed milk FA without changes in preformed milk FA yield when dietary *cis*-9 C18:1 was increased in low-producing cows. Potentially, in low-producing cows, BH-induced inhibition of de novo milk FA may have occurred, due to the decrease in de novo milk FA, without a change in the amount of preformed milk FA produced. Milk fat depression is a concern when feeding UFA, due to BH intermediates being produced that reduce milk fat synthesis in the mammary gland (Bauman et al., 2011), and although the PA+OA treatment increased production of several *trans* FA in milk fat, milk fat concentration and yield were not reduced. The increase of *trans* FA would suggest a potential mild milk fat depression situation, which could have been compensated for by the increase in dietary fatty acids, due to supplying fat supplements in the diet that would be available for the mammary gland to utilize.

The effect of fat supplements on DMI is variable, and the extent that dietary fat reduces DMI depends on the degree of saturation (Relling and Reynolds, 2007) and the type of fat supplement being fed (Rabiee et al., 2012). In general, diets higher in UFA result in a greater decrease in DMI compared with non-FA control and SFA treatments (Harvatine and Allen, 2005; Relling and Reynolds, 2007; de Souza et al., 2018), which could be attributed to increased secretion of gut peptides associated with satiety, such as CCK and glucagon-like peptide 1 (Relling and Reynolds, 2007; Bradford et al., 2008). Our study observed that overall FAT de-

Table 6. Milk fatty acid yield and content for cows fed treatment diets (n = 24)[1]

	Treatment[2]				*P*-value[4]			Contrasts[5]	
Variable	CON	PA+SA	PA+OA	SEM[3]	Trt	Prod	Trt × Prod	CON vs. FAT	PA+SA vs. PA+OA
Summation by source, g/d									
<16-carbon	436	416	396	11.8	<0.001	0.001	0.06	<0.01	0.01
16-carbon	618	676	662	20.8	<0.001	0.003	0.03	<0.01	<0.01
>16-carbon	510	532	555	16.5	<0.001	0.001	0.001	<0.01	0.01
Summation by source, g/100 g									
<16-carbon	27.9	25.6	24.5	0.24	<0.001	0.69	0.42	<0.01	<0.01
16-carbon	39.3	41.5	40.9	0.47	<0.001	0.23	0.49	<0.01	0.01
>16-carbon	32.8	32.9	34.6	0.43	<0.001	0.27	0.32	<0.01	<0.01

[1]Experimental diets fed to 24 cows (12 low-producing cows; 12 high-producing cows) in replicated 3 × 3 Latin squares with 21-d periods and balanced for carryover effects. Samples and data for production variables collected during the last 5 d of each treatment period (d 17 to 21).

[2]Treatments were CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1.

[3]Greatest SEM related to main effect of treatment.

[4]*P*-values refer to the ANOVA results for the fixed effects of treatment (Trt) and production level (Prod), and the interaction of Trt × Prod.

[5]CON vs. FAT and PA+SA vs. PA+OA contrasts tested the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1, respectively.

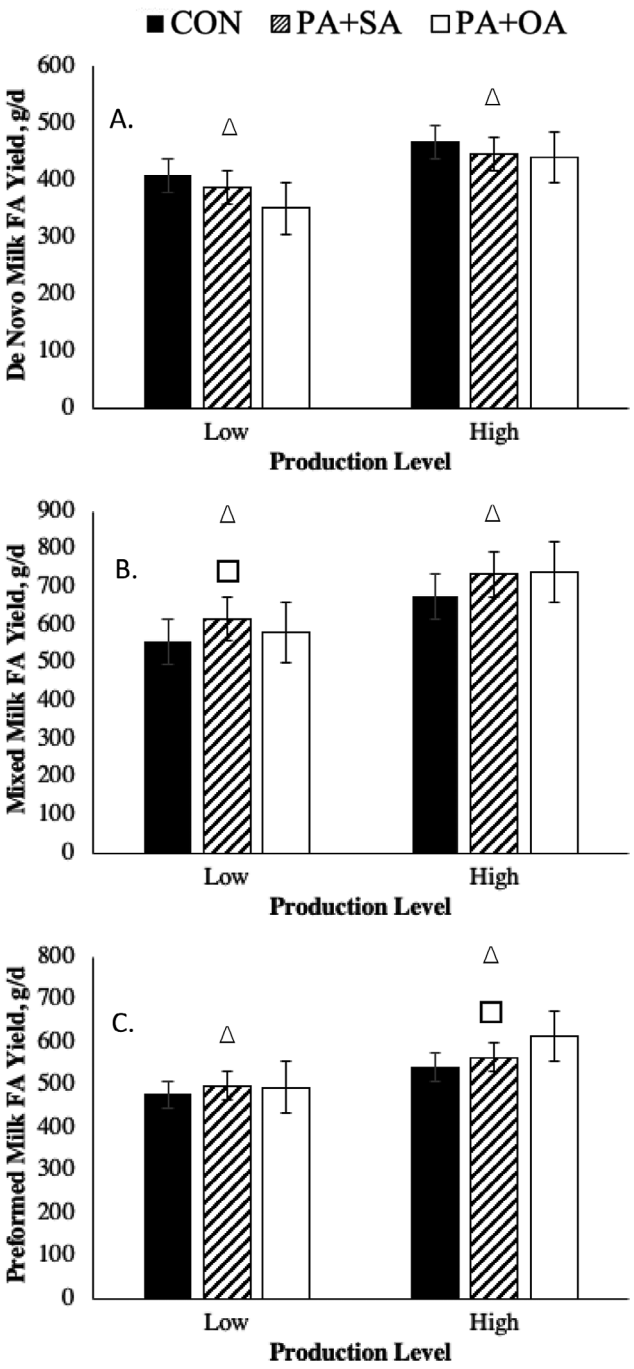

Figure 3. Effects of treatments on yields of (A) de novo (B) mixed, and (C) preformed milk FA in low-producing (42.5 ± 3.54 kg/d) and high-producing (55.8 ± 3.04 kg/d) dairy cows. Treatments were as follows: CON = no fatty acid (FA) supplementation, PA+SA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% C18:0, and PA+OA = 1.5% of a FA supplement blend providing ~60% C16:0 and 30% *cis*-9 C18:1. Significant interactions between treatments and production level were detected for the yields of de novo ($P = 0.06$), mixed ($P = 0.03$), and preformed milk FA ($P = 0.001$). Error bars represent SEM used for each individual treatment. CON vs. FAT (Δ) and PA+SA vs. PA+OA (□) contrasts ($P < 0.10$) were to test the overall effect of FA supplementation and the difference between C18:0 and *cis*-9 C18:1 within production level, respectively. See Supplemental Table S5 (https://dx.doi.org/10.5281/zenodo.4701271).

pressed DMI, and we found no difference between the PA+SA and PA+OA treatments. Varying the level of *cis*-9 C18:1 in the diet did not affect DMI (He et al., 2012), and other studies with FA blends of 60% C16:0 + 30% *cis*-9 C18:1 either had no effect on DMI (de Souza et al., 2019) or increased DMI in high-producing cows (Western et al., 2020b). In contrast, de Souza et al. (2018) reported that a blend of 45% C16:0 + 35% *cis*-9 C18:1 decreased DMI compared with blends of 80% C16:0 and 40% C16:0 + 40% C18:0, in a diet containing whole cottonseed but not in a diet containing soyhulls. Similar to results observed by de Souza et al., (2019), we did not observe an interaction between production level and FA treatment on DMI. Additional research is needed to determine whether a depression in feed intake is due to a higher intake of specific FA or UFA, and whether potential interactions with other dietary components may occur.

Total flow of FA reaching the duodenum affects FA digestibility, but differences occur among individual FA (Boerman et al., 2015). It had been reported that *cis*-9 C18:1 has greater digestibility than C16:0 and C18:0, indicating that UFA have higher digestibility than SFA (Boerman et al., 2015). Overall, the PA+OA treatment increased 16- and 18-carbon and total FA digestibility, and these results are similar to de Souza et al. (2018, 2019). In contrast, the PA+SA treatment markedly decreased 16- and 18-carbon FA digestibility. These results are supported by other experiments that have reported C18:0 supplementation decreasing 16-carbon, 18-carbon, and total FA digestibility (Boerman et al., 2017; de Souza et al., 2018; Western et al., 2020a). This decrease in FA digestion could be due to the flow of C18:0 from the rumen being several times greater than its intake, due to BH of dietary UFA, and may be related to micelle formation, due to micelles reaching their saturation point for C18:0 and excess C18:0 being retained in the hydrophobic phase (Freeman, 1969). The addition of *cis*-9 C18:1 extended the saturation point of C18:0, allowing increased amounts of C18:0 to be solubilized in the micellar phase (Freeman, 1969). These results indicate that the profile of FA reaching the intestine can affect FA digestibility and that *cis*-9 C18:1 could be a key factor in improving FA digestibility by increasing emulsification for better absorption of SFA. Further research is needed to understand the reasons for FA supplements containing C18:0 decreasing FA digestibility and how this might be overcome.

Across studies, results are variable for NDF and DM digestibility when FA are supplemented. A recent meta-analysis reported a tendency for an increase in total-tract NDF digestibility when feeding SFA supplements (Weld and Armentano, 2017). Supplementation of C16:0 has been observed to consistently increase

NDF digestibility (Rico et al., 2017; de Souza et al., 2018), likely due to dietary C16:0 being incorporated into rumen bacterial membranes instead of having to be synthesized or increased secretion of cholecystokinin increasing rumen retention time (Western et al., 2020a), whereas C18:0 did not alter NDF digestibility (Piantoni et al., 2015; Boerman et al., 2017). In our study, overall FAT increased NDF and DM digestibility in both low- and high-producing cows, but the PA+SA treatment increased these in high-producing cows. In contrast to our results, de Souza et al. (2018) and Western et al. (2020a) both observed that a blend of C16:0 + C18:0 (40:40% and 30:50%, respectively) reduced DM and NDF digestibility compared with other treatments containing 80% C16:0. Additionally, varying levels of a blend of C16:0 + *cis*-9 C18:1 did not affect NDF or DM digestibility and did not have an interaction with production level for these variables (de Souza et al., 2019). Potential causes for the increase in NDF and DM digestibility for FA supplementation found in our study are likely associated with our FA blends containing 60% C16:0 and its ability to improve NDF digestibility.

Regardless of production level, FAT reduced BW gain and no differences were detected between PA+SA and PA+OA. This is contrary to previous studies, as de Souza et al. (2018, 2019) observed that BW gain increased with increasing *cis*-9 C18:1 in FA blends. We found no difference in BCS or BCS change between treatment diets. Even though FAT overall reduced DMI, the PA+OA treatment increased feed efficiency (ECM/DMI) in high-producing cows due to the increase in ECM and a decrease in DMI. Interestingly, we did not observe a difference between treatments for insulin concentrations, which is contrary to results recently reported, with *cis*-9 C18:1 increasing insulin concentrations (de Souza et al. 2018, 2019).

CONCLUSIONS

Higher-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1, with increases in the yields of 3.5% FCM and preformed FA, compared with a treatment containing 60% C16:0 and 30% C18:0. In contrast, lower-producing dairy cows responded more favorably to a FA blend containing 60% C16:0 and 30% C18:0, with increases in the yields of 3.5% FCM, ECM, milk fat and protein, and mixed FA yield. We found no difference in BW change between the 2 production groups or between the FA treatments. Results suggest that higher-yielding cows have improved production responses when supplemented with *cis*-9 C18:1 compared with C18:0, but further research is required to validate this observation and determine mechanisms for how C18:0 and *cis*-9 C18:1 are utilized in cows with different levels of milk production.

ACKNOWLEDGMENTS

We acknowledge the Michigan Alliance for Animal Agriculture for financial support. We acknowledge L. N. Worden, C. Prom, M. Western, A. Negreiro, U. Abou Rjeily, M. Machiela, and M. Kloboves (all in the department of Animal Science, Michigan State University), and the staff of the Michigan State University Dairy Cattle Teaching and Research Center for their help and assistance in this project. The authors have not stated any conflicts of interest.

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