Effects of commercially available palmitic and stearic acid supplements on nutrient digestibility and production responses of lactating dairy cows

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ABSTRACT

We evaluated the effects of commercially available fatty acid (FA) supplements containing palmitic (C16:0) and stearic acid (C18:0) on nutrient digestibility and production responses of dairy cows. Thirty-six mid-lactation (146 ± 55 d in milk) multiparous Holstein cows were randomly assigned to twelve 3×3 balanced truncated Latin squares, with 3 treatments and 2 consecutive 35-d periods, with the final 5 d used for sample and data collection. Treatments were (1) a control diet containing no supplemental FA (CON), (2) a control diet supplemented with a commercially available C16:0 supplement (PA), and (3) a control diet supplemented with a commercially available C16:0 and C18:0 supplement (MIX). Supplements were fed at 1.5% dry matter and replaced soyhulls in CON. The statistical model included the random effect of cow nested within square and the fixed effects of treatment, period, square, and their interactions. Preplanned contrasts were (1) overall effect of FA treatments [CON vs. the average of the FA treatments (FAT); $1/2$ (PA + MIX)], and (2) effect of FA supplement (PA vs. MIX). Treatment had no effects on dry matter intake, body weight, or body weight change. Compared with CON, FAT decreased digestibilities of total FA and 18-carbon FA but did not affect dry matter and neutral detergent fiber digestibility. Compared with MIX, PA increased dry matter and neutral detergent fiber digestibilities by 3.6 and 4.8 percentage units, respectively. The PA also increased total FA and 18-carbon FA digestibilities but did not alter 16-carbon FA digestibility compared with MIX. Using a Lucas test, we estimated apparent digestibility coefficients of 0.768 and 0.553 for the PA and MIX supplements, respectively. Compared with CON, FAT

increased milk yield and tended to increase energy-corrected milk, but did not affect yield of milk fat or milk protein. The PA increased energy-corrected milk and milk fat yield but had no effect on milk protein yield compared with MIX. Our results indicate that dairy cows producing around 45 kg of milk respond better to a FA supplement enriched in C16:0 compared with a supplement containing both C16:0 and C18:0, which is likely due in part to PA increasing FA and neutral detergent fiber digestibility compared with MIX.

Key words: palmitic acid, stearic acid, digestibility, fat supplementation

INTRODUCTION

Fatty acid (FA) supplementation is commonly used to increase the energy density of diets for dairy cows. Interest in FA supplementation has increased due to benefits observed including increased milk yield and increased yields of milk components when added to rations (Rabiee et al., 2012). Palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids are the 3 main FA that are found in milk fat and adipose tissue (Palmquist, 2006; Douglas et al., 2007), as well as in most commercially available FA supplements. Research has examined the effects of feeding FA to dairy cows and differences have been reported depending on the feeding rate, production level, and FA profile (Rabiee et al., 2012). We have recently carried out research with blends of FA using commercial supplements (de Souza et al., 2018, 2019a), but research comparing commercially available FA supplements containing different amounts of C16:0 and C18:0 is limited.

A recent meta-analysis by Rabiee et al. (2012) reviewed 59 papers examining FA supplementation, but grouped all prilled SFA supplements together, not allowing assessment of individual FA or supplements differing in SFA profile. However, differences have been observed between C16:0 and C18:0 supplementation (e.g., Rico et al., 2014b; de Souza et al., 2018). Supplementation of C16:0 increases milk fat concentration and yield and NDF digestibility in post-peak (Piantoni et al., 2013; Mathews et al., 2016; de Souza et al., 2018)

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and early-lactation cows (de Souza et al., 2019a). Rico et al. (2017) fed increasing levels of a C16:0 supplement to dairy cows (from 0.75 to 2.25% diet DM) and observed positive responses on production up to 1.5% with only small decreases in FA digestibility. In contrast, Boerman et al. (2017) fed similar levels of a C18:0 supplement (from 0.80 to 2.30% diet DM) and observed that FA digestibility markedly decreased as FA intake increased and C18:0 supplementation had no effect on milk production. Digestibility of FA decreased in both studies, but supplementation with C16:0 decreased FA digestibility to a much lesser degree than supplementation with C18:0. In support, a meta-analysis by Boerman et al. (2015a) reported intestinal digestibility of C18:0 across 15 studies and concluded C18:0 flow through the duodenum reduces digestibility of several FA.

Recently, a review by Loftén et al. (2014) suggested that feeding a combination of C16:0 and C18:0 in a FA supplement is needed to optimize their utilization for milk production and overall performance of the dairy cow. In contrast, in a short-term study (21-d periods), de Souza et al. (2018) found no support for this theory because feeding a FA blend (40% C16:0 and 40% C18:0) reduced nutrient digestibility and NE_L intake and animal performance compared with treatments with other blends of FA, including a blend containing 80% C16:0, 10% *cis*-9 C18:1, and 5% C18:0. Also, Rico et al. (2014b) compared diets supplemented with C16:0 or C18:0 for 21 d in post-peak cows and observed that C16:0 increased milk fat and 3.5% FCM but had no effect on BW or DMI compared with C18:0. Because Rico et al. (2014b) evaluated highly enriched C16:0 and C18:0 supplements (>97% individual FA), this raises the question of whether responses to C16:0 supplements are similar when compared with commercially available mixed SFA supplements containing both C16:0 and C18:0.

Therefore, the objective of our study was to determine the effects of commercially available C16:0 and mixed SFA supplements on nutrient and gross energy (GE) digestibility, and production responses of lactating dairy cows across longer periods. Our hypothesis was that a C16:0 supplement will increase milk production and will not affect BW gain, whereas a mixed SFA supplement will decrease FA digestibility and milk production.

MATERIALS AND METHODS

Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan

State University. Thirty-six mid-lactation (146 ± 55 DIM) multiparous Holstein cows from the Michigan State Dairy Teaching and Research Center were randomly assigned to twelve 3×3 balanced truncated Latin squares, the truncation occurring from the removal of the third period in each of the squares, and each period being a consecutive 35 d in length (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-17242>). The study was completed from June through August 2017. All animals received a common diet with no FA supplementation during a 10-d preliminary period to obtain baseline values. Cows were blocked by production level based on the data collected during the preliminary period and assigned to 1 of 3 treatment sequences. Treatments were (1) a control diet containing no supplemental FA (CON), (2) a control diet supplemented with a commercially available C16:0 supplement (PA, Spectrum Fusion; Perdue Agribusiness, Salisbury, MD; supplement is a blend containing 82% prills and 18% Ca-salts), and (3) a control diet supplemented with a commercially available C16:0 and C18:0 supplement (MIX, Energy Booster 100, Milk Specialties Global, Eden Prairie, MN; supplement is a prill). Supplements were added at 1.5% of diet DM, replacing 1.5% of soyhulls in the diet. Characterization and FA profiles of the supplements are shown in Table 1. Truncated Latin square designs have been used previously in experiments in dairy science (Clark and Armentano, 1999; Weiss et al., 2013; de Souza et al., 2019a). For our truncated Latin square design with 3×2 treatments and periods, multiples of 12 cows were required to balance treatment sequences; therefore, each cow was assigned to 2 treatments in 2 different periods. We used this truncated design to allow longer experimental periods for adaptation and data measurements compared with more traditional 2- to 3-wk periods for complete Latin squares.

The ingredient and nutrient composition of the diets fed as TMR were formulated to meet the requirements of the average cow in the group (NRC, 2001; Table 2). Dry matter concentration was determined twice weekly for forages, and diets were adjusted accordingly. All cows remained in the same tiestall throughout the experiment. Cows were milked twice per day (0300 and 1400 h). Access to feed was blocked from 0800 to 1000 to allow for collection oforts and offering feed. Cows were fed at 115% of expected intake at 1000 h daily. Water was available ad libitum in each stall, which was bedded with sawdust and cleaned twice per day.

Data and Sample Collection

Sampling and data collection for nutrient digestibility, plasma metabolites and hormones, and production

Table 1. Composition of fatty acid (FA) supplements fed during treatment periods¹

Item	C16:0 + C18:0 supplement ²	C16:0 supplement ³
Melting point, °C	57.3	74.8
Iodine value	11.4	7.60
Free FA, %	87.3	80.5
Glycerol, %	1.65	0.01
FA profile of each treatment, g/100 g of FA		
C14:0	2.28	0.66
C16:0	33.1	84.3
C18:0	53.3	4.06
<i>cis</i> -9 C18:1	5.17	8.70
<i>cis</i> -9, <i>cis</i> -12 C18:2	0.65	1.58
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.01	0.04

¹Average (n = 2) composition of FA supplements based on samples taken during the collection period.²Energy Booster 100; Milk Specialties Global, Eden Prairie, MN.³Spectrum Fusion; Perdue Agribusiness, Salisbury, MD.**Table 2.** Ingredient and nutrient composition of treatment diets

Item	Treatment ¹		
	CON	MIX	PA
Ingredient, % of DM			
Corn silage	36.8	36.8	36.8
Wheat straw	6.00	6.00	6.00
Ground corn	17.9	17.9	17.9
High-moisture corn	4.08	4.08	4.08
Soybean meal	16.7	16.7	16.7
Soyhulls	10.9	9.37	9.37
Cottonseed	2.57	2.57	2.57
Protein supplement ²	1.15	1.15	1.15
C16:0 FA supplement ³	0.00	0.00	1.49
C16:0 + C18:0 FA supplement ⁴	0.00	1.49	0.00
Mineral and vitamin mix ⁵	3.94	3.94	3.94
Nutrient composition, % of DM			
NDF	30.9	29.9	29.9
Forage NDF	19.1	19.1	19.1
CP	16.9	16.6	16.6
Starch	27.0	26.8	26.8
FA	2.71	4.22	4.29
16:0	0.46	0.97	1.96
18:0	0.09	0.93	0.17
<i>cis</i> -9 18:1	0.50	0.59	0.55
<i>cis</i> -9, <i>cis</i> -12 18:2	1.46	1.42	1.41
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.10	0.09	0.10

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).²Protein supplement (Perdue Agribusiness, Salisbury, MD).³Palmitic acid supplement (Spectrum Fusion, Perdue Agribusiness). Supplement is a blend including most of the SFA as a prill and most of the UFA as a Ca salt. The supplement contained (g/100 g of fatty acids) 0.66 of C14:0, 84.3 of C16:0, 4.06 of C18:0, 8.70 of *cis*-9 C18:1, and 94.4% total fatty acids. FA = fatty acid.⁴Palmitic and stearic prill FA supplement (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN). The supplement contained (g/100 g of fatty acid) 2.28 of C14:0, 33.1 of C16:0, 53.3 of C18:0, 5.17 of *cis*-9 C18:1, and 89.2% total fatty acids.⁵Vitamin and mineral mix contained 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, sodium selenite, iodine (ethylenediamine dihydroiodide), cobalt carbonate, vitamin E, vitamin A, and vitamin D.

variables occurred during the last 5 d of each treatment period (d 31 to 35). During sampling periods, samples of all diet ingredients and orts from each cow were collected daily and composited by period for analysis. Fecal samples were collected every 15 h during the sampling period to total 8 samples per cow per period. The 15-h interval over 5 d simulated sampling every 3 h over a 24-h period. Feces were stored in a sealed plastic cup at −20°C until dried, ground, and composited per cow per period. One blood sample was collected in the middle of the sampling period (d 33, 0700 h). Blood was stored on ice until centrifugation at 3,000 × *g* for 15 min at 4°C (within 30 min of sample collection). Plasma was transferred into microcentrifuge tubes and stored at −20°C. Milk yield was recorded, and 2 milk samples collected at each milking. One aliquot was collected in a sealed tube with preservative (Bronopol tablet, D&F Control Systems, San Ramon, CA) and stored at 4°C for milk component analysis. The second aliquot was stored without preservative at −20°C until analyzed for FA composition.

Body weight was measured 3 times per week throughout the study, following the afternoon milking. Body weight change was calculated according to Boerman et al. (2015b). Body condition score was determined on the last day of the preliminary period as well as the last day of each treatment period by 3 trained investigators on a 5-point scale (0.25-point increments; Wildman et al., 1982).

Sample Analysis

Feed ingredients, orts, and fecal samples were dried at 55°C in a forced-air oven for 72 h for DM analysis. 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). Indigestible NDF was used as an internal marker to predict fecal output to determine apparent total-tract digestibility (Cochran et al., 1986). Indigestible NDF was estimated as NDF after 240-h in vitro fermentation (Goering and Van Soest, 1970) and total-tract digestibility was calculated using total-tract disappearance. Gross energy was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL). Calculations for intake of ME and NE were calculated using digestible energy (DE) to ME and ME to NE according to NRC (2001). The commercial FA supplements were analyzed for melting point (method Cc 1-25; AOCS, 2013), iodine value (method Cd 1d-92; AOCS, 2013), and percentage of free FA (method Ca 5a-40; AOCS, 2013) by Eurofins Global Inc. (Des Moines, IA). Plasma nonesterified fatty acids (NEFA) and BHB were analyzed using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing). Plasma insulin concentrations were determined by ELISA (Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden). Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990, method 972.160) by the Michigan Herd Improvement Association (Universal Lab Services, Grand Ledge, MI). Yields of 3.5% FCM and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period. Milk samples for FA analysis were composited based on milk fat yield (d 31 to 35 of each treatment period). Milk lipids were extracted and FAME prepared according to Lock et al. (2013). Yields of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentration to determine yield on a mass basis using the molecular weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Digestibility of supplemental fat was estimated with a Lucas test. The intake of FA from the basal diet was subtracted from that in each supplemented diet to calculate the supplemental FA intake. Fecal output of undigested basal FA was estimated using FA digestibility measured when cows were fed the control diet. Fecal output of basal FA was subtracted from fecal output of total FA when cows were fed the supplemented diets. Digestibility of supplemental fat was estimated with a Lucas test by regressing supplemental FA intake by supplemental FA absorbed. The slope of each regression is the estimated apparent digestibility coefficient of each fat supplement.

Energy intakes and balance were calculated using equations (NRC, 2001) according to Harvatine and Allen (2006). Digestible energy intake = GE intake $\times$ GE digestibility; NE_L intake was calculated from DE according to NRC (2001). Milk energy output (Mcal/d) was calculated according to NRC (2001) as milk energy output (Mcal/d) = $[9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}]$, where each component was based on the average output of a cow during the sampling period. Energy for maintenance (Mcal/d) was calculated as $0.08 \text{ Mcal/kg} \times \text{BW (kg)}^{0.75}$ and energy balance (Mcal/d) determined as NE_L intake (Mcal/d) – milk NE_L (Mcal/d) – NE_L maintenance requirement (Mcal/d) (NRC, 2001).

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:

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

where Y_{ijkl} = the dependent variable, μ = the overall mean, S_i = the fixed effect of square ($i = 1$ to 12), $A_j(S_i)$ = the random effect of cow within square ($j = 1$ to 3), P_k = the fixed effect of period ($k = 1$ to 2), T_l = the fixed effect of treatment ($l = 1$ to 3), $P_k \times T_l$ was the fixed effect of the interaction between period and treatment, and e_{ijkl} = the residual error. Normality of the residuals was checked with the normal probability, box plots, and homogeneity of variances with plots of residuals versus predicted values. Two orthogonal contrasts were evaluated: (1) the overall effect of FA supplements {CON vs. the average of the FA treatments (FAT) $[1/2 (\text{PA} + \text{MIX})]$ }, and (2) the effect of PA versus mixed SFA supplements (PA vs. MIX). Contrasts were declared significant at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. A Lucas test was performed using regression analysis. The CONTRAST statements were used for testing whether the slope parameters differed among the 2 fat supplements used. All data were expressed as least squares means and standard error of the means, unless otherwise specified.

RESULTS

Nutrient Intake and Total-Tract Digestibility

Compared with CON, FAT did not affect DMI ($P = 0.83$), NDF intake ($P = 0.42$), DM digestibility ($P = 0.53$), or NDF digestibility ($P = 0.11$, Table 3). The FAT increased total FA intake ($P < 0.01$), 16-carbon

Table 3. Nutrient intake and nutrient digestibility for cows fed treatment diets (n = 36)

Variable	Treatment ¹			SEM	Contrast ²	
	CON	MIX	PA		CON vs. FAT	MIX vs. PA
Intake, kg/d						
DM	30.6	30.4	30.6	0.49	0.90	0.55
NDF	9.43	9.61	9.43	0.15	0.51	0.26
Intake, g/d						
Total fatty acids	866	1,360	1,340	19.2	<0.01	0.32
16-Carbon	135	300	510	6.11	<0.01	<0.01
18-Carbon	671	971	772	12.6	<0.01	<0.01
Digestibility, %						
DM	64.3	63.0	66.6	0.67	0.53	<0.01
NDF	39.1	38.3	43.1	0.92	0.12	<0.01
Total fatty acids	76.7	67.6	76.3	1.00	<0.01	<0.01
16-Carbon	74.3	68.0	69.0	1.18	<0.01	0.55
18-Carbon	78.3	67.2	82.1	1.00	<0.01	<0.01
Absorbed, g/d						
Total fatty acids	666	919	1,021	16.6	<0.01	<0.01
16-Carbon	100	202	354	5.52	<0.01	<0.01
18-Carbon	528	648	628	11.3	<0.01	0.09

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).

²Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + MIX)] of the FA treatments (FAT); and MIX versus PA: the comparison between the PA and MIX treatments.

FA intake ($P < 0.01$), and 18-carbon FA intake ($P < 0.01$) compared with CON, but decreased total ($P < 0.01$), 16-carbon ($P < 0.01$), and 18-carbon FA digestibility ($P < 0.01$). The FAT increased absorbed total ($P < 0.01$), 16-carbon ($P < 0.01$), and 18-carbon FA ($P < 0.01$) compared with CON.

Total FA intake was not different between PA and MIX ($P = 0.32$, Table 3). Compared with MIX, PA increased 16-carbon ($P < 0.01$) and decreased 18-carbon FA intake ($P < 0.01$). No difference was observed between PA and MIX for 16-carbon digestibility ($P = 0.55$), but PA increased the digestibility of DM ($P < 0.01$), NDF ($P < 0.01$), total FA ($P < 0.01$), and 18-carbon FA ($P < 0.01$) compared with MIX. The PA increased total ($P < 0.01$) and 16-carbon FA ($P < 0.01$) absorption compared with MIX, and tended to decrease 18-carbon FA absorption ($P = 0.09$, Table 3). By using a Lucas test, we estimated a ~20 percentage point increase for the digestibility of total FA in the PA supplement compared with the MIX supplement (77 vs. 55%, respectively, Figure 1).

Energy Intake, Output, and Digestibility

No difference was observed between CON and FAT for DE intake ($P = 0.19$), ME intake ($P = 0.19$), NE_L intake ($P = 0.19$), GE digestibility ($P = 0.75$), milk energy output ($P = 0.11$), energy for maintenance (P

$= 0.96$), and energy balance ($P = 0.49$, Table 4). However, compared with MIX, PA increased DE intake ($P = 0.05$), ME intake ($P = 0.04$), NE_L intake ($P = 0.03$), GE digestibility ($P < 0.01$), milk energy output ($P = 0.05$), and energy balance ($P = 0.03$).

Production Results

Compared with CON, FAT increased milk production ($P < 0.01$) and tended to increase 3.5% FCM ($P = 0.07$) and ECM ($P = 0.08$, Table 5). The FAT decreased milk protein concentration ($P = 0.03$) and tended to decrease milk fat concentration ($P = 0.09$). We observed no differences in BW, BW change, BCS, or BCS change between CON and FAT.

No differences were observed between PA and MIX for milk yield ($P = 0.35$, Table 5). However, compared with MIX, PA increased 3.5% FCM yield ($P = 0.01$) 2.1 kg/d, ECM yield ($P = 0.03$) 1.9 kg/d, fat yield ($P < 0.01$) 0.13 kg/d, and fat concentration ($P < 0.01$) 0.28 percentage units. No differences were observed for protein concentration ($P = 0.87$), protein yield ($P = 0.47$), lactose concentration ($P = 0.83$), or lactose yield ($P = 0.49$). A tendency was observed for PA to decrease BCS compared with MIX ($P = 0.08$), but no differences were observed between PA and MIX for BW ($P = 0.44$), BW change ($P = 0.36$), or BCS change ($P = 0.21$, Table 5).

Milk Fatty Acid 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. Compared with CON, FAT decreased the concentration of de novo milk FA and increased the concentration of mixed milk FA ($P < 0.01$) but had no effect on preformed milk FA (Table 6). On a yield basis, FAT decreased de novo milk FA ($P < 0.01$), increased mixed milk FA ($P < 0.01$), but had no effect on preformed milk FA ($P = 0.21$).

Compared with MIX, PA decreased the concentration of de novo milk FA ($P < 0.01$) and preformed milk FA ($P < 0.01$) and increased the concentration of mixed milk FA ($P < 0.01$, Table 6). The increase in mixed milk FA was predominantly due to the increased concentration of C16:0 in milk fat for PA ($P < 0.01$, Supplemental Table S1; <https://doi.org/10.3168/jds.2019-17242>).

Compared with MIX, PA decreased concentrations of C18:0 ($P < 0.01$) and *cis*-9 C18:1 ($P < 0.01$) in milk fat. On a yield basis, PA increased mixed milk FA ($P < 0.01$) and tended to decrease preformed milk FA compared with MIX ($P = 0.06$, Table 6). Treatment had no effect on the yield of de novo milk FA ($P = 0.67$). The PA increased the yield of C16:0 ($P < 0.01$) and had no effect on C18:0 ($P = 0.13$) or *cis*-9 C18:1 ($P = 0.12$) compared with the MIX treatment (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-17242>).

Blood Metabolites

The FAT had no effect on plasma insulin or BHB ($P = 0.23$ and $P = 0.15$, respectively), but increased NEFA concentration ($P < 0.01$, Table 7). Compared with MIX, PA increased NEFA ($P < 0.01$), but no difference was observed between PA and MIX for insulin or BHB ($P < 0.96$ and $P < 0.13$).

Figure 1. Lucas test to estimate the apparent fatty acid (FA) digestibility of a supplement containing C16:0 and C18:0 (MIX) and a supplement enriched in C16:0 (PA). To estimate the digestibility of the supplements, the intake of FA from the basal diet was subtracted from that in each supplemented diet to calculate supplemental FA intake. Fecal output of undigested basal FA was estimated using FA digestibility measured when cows were fed the control diet. Fecal output of basal FA was subtracted from fecal output of total FA when cows were fed the supplemented diets. Estimated slope for PA was greater than MIX ($P < 0.05$). The slopes (i.e., apparent digestibility of supplemental fat) were 0.768 ± 0.021 and 0.553 ± 0.023 for PA and MIX, respectively, and intercepts were not different from zero ($P > 0.65$).

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

Variable	Treatment ¹			SEM	Contrast ²	
	CON	MIX	PA		CON vs. FAT	MIX vs. PA
Energy digestibility, %	64.5	62.6	66.9	0.70	0.75	<0.01
Energy intake, Mcal/d						
DE ³	86.2	86.5	91.6	2.15	0.19	0.05
ME ⁴	73.5	73.6	78.7	1.93	0.19	0.04
NE _L ⁵	45.9	45.9	49.5	1.25	0.19	0.03
Energy output, Mcal/d						
Milk ⁶	30.7	30.9	32.0	0.86	0.11	0.05
Maintenance ⁷	11.2	11.4	11.3	0.13	0.96	0.37
Energy balance ⁸	4.05	3.58	6.15	1.11	0.49	0.03

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).

²Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + MIX)] of the FA treatments (FAT); and MIX versus PA: the comparison between the PA and MIX treatments.

³Digestible energy (DE) intake = gross energy intake (Mcal/d) × gross energy digestibility.

⁴Metabolizable energy intake was calculated from DE according to NRC (2001).

⁵Net energy of lactation intake was calculated from DE through ME according to NRC (2001).

⁶Milk NE_L (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)] (NRC, 2001).

⁷NE_L maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg)^{0.75} (NRC, 2001).

⁸Energy balance (Mcal/d) = NE_L intake (Mcal/d) – milk NE_L (Mcal/d) – NE_L maintenance requirement (Mcal/d).

DISCUSSION

Fatty acid supplements have commonly been fed to dairy cows to increase energy density of the ration and support milk production; however, limited research is

available comparing the effect of commercially available supplements differing in FA profile on production and nutrient digestibility responses. C16:0, C18:0, and *cis*-9 C18:1 are the predominant FA found in commercial FA supplements. These 3 FA are also the most

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

Variable	Treatment ¹			SEM	Contrast ²	
	CON	MIX	PA		CON vs. FAT	MIX vs. PA
Milk yield, kg/d						
Milk	43.1	44.8	45.7	1.36	0.01	0.35
3.5% FCM ³	44.1	43.9	46.2	1.21	0.07	0.01
ECM ⁴	44.8	44.5	46.4	1.21	0.08	0.03
Milk composition						
Fat, kg/d	1.55	1.52	1.65	0.05	0.19	<0.01
Fat, %	3.63	3.41	3.69	0.10	0.09	<0.01
Protein, kg/d	1.43	1.46	1.44	0.04	0.32	0.47
Protein, %	3.32	3.27	3.26	0.05	0.03	0.87
Lactose, kg/d	2.10	2.16	2.14	0.07	0.08	0.49
Lactose, %	4.79	4.80	4.79	0.03	0.84	0.83
BW, kg	744	738	742	11.7	0.34	0.44
BW change, kg/d	0.42	0.35	0.67	0.25	0.76	0.36
BCS	3.51	3.58	3.47	0.07	0.90	0.08
BCS change	0.09	0.09	0.14	0.02	0.53	0.21

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).

²Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + MIX)] of the FA treatments (FAT); and MIX versus PA: the comparison between the PA and MIX treatments.

³3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

⁴ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

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

Variable	Treatment ¹			SEM	Contrast ²	
	CON	MIX	PA		CON vs. FAT	MIX vs. PA
Summation by source, ³ g/100 g						
De novo	27.8	26.3	24.4	0.39	<0.01	<0.01
Mixed	37.5	36.8	42.5	0.49	<0.01	<0.01
Preformed	34.7	36.9	33.1	0.55	0.47	<0.01
Summation by source, ³ g/d						
De novo	404	380	376	14.8	<0.01	0.67
Mixed	551	525	649	21.0	<0.01	<0.01
Preformed	499	524	500	14.2	0.21	0.06

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).

²Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + MIX)] of the FA treatments (FAT); and MIX versus PA: the comparison between the PA and MIX treatments.

³De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Supplemental Tables S1 and S2, respectively (<https://doi.org/10.3168/jds.2019-17242>).

prevalent in milk fat, averaging ~50 to 80% of milk FA (Jensen, 2002). Recent research determined the effects of differing ratios of these 3 FA on nutrient digestibility, nutrient partitioning, and production (de Souza et al., 2018). The FA ratios that were used by de Souza et al. (2018) were similar to commercial products but were made with blends of different FA supplements to achieve predetermined ratios. Most of our research, and work by others, has been conducted using changeover design experiments (i.e., crossover and Latin squares) with production and metabolic responses evaluated during short-term feeding (maximum of 21-d feeding periods). This raises a question about the consistency of the response under long-term conditions and the effects of these FA profiles on BW changes and energy partitioning. The goal of our study was to use commercially available products and evaluate their effects on nutrient digestibility and production measurements. Our study was completed during the summer months from June to August and consisted of 35-d periods, which is longer than earlier FA supplementation research.

Although previous research indicates that the amount and profile of FA supplements affect feed intake (Rabiee et al., 2012), there is substantial variation in DMI responses when supplementing different FA supplements. Rico et al. (2014b) supplemented cows with nearly pure C16:0 and C18:0 (>97% total FA) at 2% of diet DM, and they found no effect on DMI when comparing the two. Similarly, de Souza et al. (2018) observed no differences in DMI in post-peak lactating cows fed different ratios of C16:0 and C18:0 at 1.5% of DMI compared with cows fed a control diet with no supplemental FA. Consistent with these findings, we also observed no difference in DMI for CON versus FAT or for PA versus MIX when supplemental FA were included in the diet at 1.5% diet DM. Among the different FA supplements, previous meta-analyses have observed that Ca-salts of palm fatty acid distillate (PFAD) reduced DMI (Allen, 2000; Rabiee et al., 2012). The hypophagic effect of feeding fat seems to be more pronounced for unsaturated than for SFA supplements (Harvatine and Allen, 2006), with DMI decreasing linearly as the degree of

Table 7. Plasma insulin and metabolites for cows fed treatment diets (n = 36)

Variable	Treatment ¹			SEM	Contrast ²	
	CON	MIX	PA		CON vs. FAT	MIX vs. PA
Insulin, µg/L	0.77	0.82	0.83	0.05	0.23	0.96
NEFA, ³ mEq/L	0.09	0.10	0.11	0.004	<0.01	0.03
BHB, mg/dL	6.71	6.14	6.58	0.27	0.15	0.13

¹Treatments were (1) CON (no supplemental fat), (2) MIX (1.5% of diet DM of a C16:0 + C18:0 supplement), and (3) PA (1.5% of diet DM of a C16:0 supplement).

²Pre-planned contrasts included CON versus FAT: the comparison between the control treatment (CON) and the average [1/2 (PA + MIX)] of the FA treatments (FAT); and MIX versus PA: the comparison between the PA and MIX treatments.

³NEFA = nonesterified fatty acids.

unsaturation increases (Drackley et al., 1992; Pantoja et al., 1994) and the chain length of FA infused into the abomasum increases (Drackley et al., 1992). The more pronounced decrease in DMI for UFA is likely mediated in part by increased secretion of gut peptides related to satiety (i.e., cholecystokinin and GLP-1; Bradford et al., 2008). In our study, although the FA supplement used in the PA treatment is a blend containing Ca-Salts, we did not observe differences in DMI. Possible explanations for these differences include the level of UFA in the supplement compared with Ca-salts of PFAD, the amount of UFA reaching the small intestine, and possibly the use of different raw materials used in the manufacturing of supplements (e.g., PFAD vs. fractionated FA). Further research is needed to determine whether a higher amount of UFA or the physical form of the supplement is related to reduced DMI.

A recent meta-analysis observed a tendency for SFA supplements to increase total-tract NDF digestibility (Weld and Armentano, 2017). Increases in NDF digestibility have been observed in studies supplementing C16:0 compared with both control (no FA supplementation) and diets supplemented with other FA (Piantoni et al., 2013; de Souza et al., 2018; de Souza and Lock, 2018). The C16:0-enriched supplements (C16:0 ranging from 81 to 98% total FA) increased NDF digestibility in these studies from 3.3 to 5.0 percentage units. In contrast, compared with control diets (no FA supplementation), C18:0-enriched supplements (C18:0 ranging from 93 to 98% total FA) did not alter NDF digestibility (Piantoni et al., 2015; Boerman et al., 2017). In our study, there was no difference in DM or NDF digestibility when comparing CON to FAT, but we observed an increase in both DM and NDF digestibility for PA compared with MIX. The lack of difference between CON and FAT was due to a numeric decrease in NDF digestibility for the MIX treatment compared with control and a 4.0%-unit increase over control with the PA treatment. These responses are similar to those observed by de Souza et al. (2018) where he compared an 80% C16:0 blend and a 40% C16:0 + 40% C18:0 blend. Piantoni et al. (2013) did not observe a difference in DMI and suggested that increases in NDF digestibility with C16:0 supplementation could be due to the increased secretion of cholecystokinin, leading to an increased retention time in the rumen. The increase in NDF digestibility with C16:0 supplementation may also be due to sparing of carbon for ATP usage during fermentation if dietary C16:0 could be included into rumen bacterial membranes instead of having to be synthesized (Vlaeminck et al., 2006; Hackmann and Firkens, 2015). Importantly, we did not observe an increase in NDF digestibility with the mixed SFA

supplement in our current study. Due to these findings, increases in NDF digestibility appear to be specific to C16:0 in SFA supplements. Further research is required to determine the mechanisms for increased NDF digestibility when C16:0 is supplemented.

Frequently, FA supplementation has decreased total FA digestibility, although responses are variable based on which FA are supplemented (Boerman et al., 2015a; Rico et al., 2017; de Souza et al., 2018). However, Rico et al. (2014a) supplemented a C16:0-enriched supplement and reported increases in total and 16-carbon FA digestibilities in low-producing cows. In a meta-analysis, Boerman et al. (2015a) observed that as the flow of C18:0 reaching the duodenum increased, the digestibility of C18:0 linearly decreased compared with control diets with no added FA. In our study, FAT decreased the digestibility of total, 16-carbon, and 18-carbon FA compared with CON, but PA increased total and 18-carbon digestibility compared with MIX. The decrease in total FA digestibility when comparing CON and FAT was driven by the substantial decrease (15 percentage units) in 18-carbon digestibility for MIX. The increase in FA digestibility for PA compared with MIX resulted in an increase of ~100 g/d total FA absorbed despite the supplements being fed at the same inclusion rate. Although the exact mechanisms for the reduction in FA digestibility as FA intake increases are unknown, potential causes have been suggested and include competition for absorption sites, and limitation in emulsification (Drackley, 2000). It is possible that the decrease observed in FA absorption is limited by the supply of lysolecithin, an amphiphile and natural emulsifier that assists in the formation of micelles (Freeman, 1969). The differences observed between the SFA supplements suggest specific FA or the profile of the FA in the supplement affect FA digestibility. Also, the supplement used in the PA treatment contained higher amount of UFA than the MIX supplement. Although total flow of FA at the duodenum affects FA digestibility (Boerman et al., 2015a), previous studies indicate that the profile of FA entering the duodenum is a critical factor affecting FA digestibility (Rico et al., 2017; de Souza et al., 2018). Unsaturated FA usually have higher digestibility than SFA, 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). Further research is needed to examine the mechanisms related to FA absorption and its limitations.

Most research reporting energy intakes use predicted values that are generated through calculations from dietary ingredients and observations have mostly been

with cows during early lactation. Considering the high variability in nutrient digestibility among cows (Piantoni et al., 2013) and the observed effects that individual FA have on the digestibility of FA and other nutrient fractions, estimating energy intake only from dietary composition is insufficient for the calculation of energy intake and energy balance. Moallem et al. (2007) fed a supplement enriched in C16:0 and C18:0 and reported no difference in predicted energy intake compared with no supplemental FA. However, in our current study, we used bomb calorimetry to determine energy in feed, feces, and orts to calculate energy digestibility and DE intake. We observed no difference between CON and FAT for GE digestibility or DE intake; however, PA increased both variables compared with MIX. Actual NE_L intake from our bomb calorimetry data and NRC (2001) conversion was 45.9, 45.9, and 49.5 Mcal/d for CON, MIX, and PA, respectively. However, predicted NE_L intake from dietary composition using NRC (2001) model was 48.3, 49.9, and 50.3 Mcal/d for CON, MIX, and PA, respectively. Similar to our results, de Souza et al. (2019b) reported that predicting NE_L from dietary composition using the NRC model would estimate that C16:0 supplementation (1.5% diet DM) increased NE_L intake by only 1 Mcal/d, whereas the actual calculated increase in NE_L intake was 2.5 Mcal/d. Bomb calorimetry provides energy values instead of predicted values from calculations and our results clearly show the challenge of relying on predicted values. Our results showing improved NDF and FA digestibility and increased energy intake with PA compared with MIX does not support the hypothesis that feeding a combination of C16:0 and C18:0 is most beneficial for performance measures (Loften et al., 2014).

In our study, overall compared with CON, FAT increased milk yield by 2.1 kg/d as well as 3.5% FCM and ECM yields. However, the increases in 3.5% FCM and ECM yield with FAT were only driven by increases with PA. These results are consistent with de Souza and Lock (2018) where a similar increase in ECM yield was seen when comparing C16:0 supplementation (1.5% diet DM) to a control diet across 10 wk of supplementation. Short-term feeding studies with increased levels of palmitic acid have shown similar results (Rico et al., 2014a; de Souza et al., 2018). We observed no differences in BW change or BCS between treatments. Similarly, de Souza et al. (2018) observed no differences between feeding a FA blend with high content of C16:0 (80% C16:0, 10% *cis*-9 C18:1, and 5% C18:0) and a FA blend with a combination of C16:0 and C18:0 (40% C16:0, 40% C18:0, and 10% *cis*-9 C18:1) on BW or BCS. Similarly, increased plasma NEFA concentra-

tions have been observed when adding SFA to dairy rations (Choi et al., 2000; Piantoni et al., 2013). We did see increases in plasma NEFA concentrations between CON and FAT and PA and MIX, but no differences in BW or BCS were observed, meaning this is not due to mobilization of body reserves (Rico et al., 2014b) and most likely due to more plasma triglyceride FA absorbed with PA.

It is well established that feeding FA supplements can alter the FA profile of milk. A recent review by Dorea and Armentano (2017) indicated that C16:0 supplementation increased total milk FA. This increase is mainly due to the increase in mixed source (16-carbon) FA. Similarly, we found that PA increased the secretion of mixed FA compared with MIX. Likewise, compared with MIX, PA decreased preformed yields and decreased *de novo* and preformed concentrations of milk FA. Additionally, PA increased yield of C4:0 in milk, which has been seen previously (Piantoni et al., 2013; Lock et al., 2013; Rico et al., 2014b), and likely is linked to mechanisms in the mammary gland to regulate milk fluidity as more longer-chain FA enter the mammary gland (Barbano and Sherbon, 1980).

CONCLUSIONS

Overall, milk yield was increased with supplemental fat compared with control. Compared with the mixed SFA supplement, the C16:0-enriched supplement increased NDF, FA, and GE digestibility, DE intake, ECM, milk fat yield all while having no effect on DMI or BW gain. Therefore, our results indicate that dairy cows producing around 45 kg of milk responded better to a FA supplement enriched in C16:0 compared with a supplement containing both C16:0 and C18:0.

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