Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows

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ABSTRACT

The effect of a high-palmitic acid fat supplement was tested in 12 high-producing (mean = 42.1 kg/d) and 12 low-producing (mean = 28.9 kg/d) cows arranged in a replicated 3 × 3 Latin square design. Experimental periods were 21 d, with 18 d of diet adaptation and 3 d of sample collection. Treatments were (1) control (no supplemental fat), (2) high-palmitic acid (PA) supplement (84% C16:0), and (3) Ca salts of palm fatty acid (FA) supplement (Ca-FA). The PA supplement had no effect on milk production, but decreased dry matter intake by 7 and 9% relative to the control in high- and low-producing cows, respectively, and increased feed efficiency by 8.5% in high-producing cows compared with the control. Milk fat concentration and yield were not affected by PA relative to the control in high- or low-producing cows, although PA increased the yield of milk 16-C FA by more than 85 g/d relative to the control. The Ca-FA decreased milk fat concentration compared with PA in high-, but not in low-producing cows. In agreement, Ca-FA dramatically increased milk fat concentration of *trans*-10 C18:1 and *trans*-10,*cis*-12 conjugated linoleic acid (>300%) compared with PA in high-producing cows, but not in low-producing cows. No effect of treatment on milk protein concentration or yield was detected. The PA supplement also increased 16-C FA apparent digestibility by over 10% and increased total FA digestibility compared with the control in high- and low-producing cows. During short-term feeding, palmitic acid supplementation did not increase milk or milk fat yield; however, it was efficiently absorbed, increased feed efficiency, and increased milk 16-C FA yield, while minimizing alterations in ruminal biohydrogenation commonly observed for other unsaturated fat supplements. Longer-term experiments will be necessary to determine the effects on energy balance and changes in body reserves.

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

INTRODUCTION

Fat supplements are commonly fed to dairy cows to increase dietary energy density, increase energy intake, and maximize milk yield without the risks associated with feeding excessive fermentable carbohydrates (Jenkins and McGuire, 2006). The ideal fat supplement has no effect on ruminal fermentation or DMI, is highly digestible in the intestine, and increases milk and milk component yields. However, some fat supplements decrease fiber digestibility (Jenkins and Palmquist, 1984), decrease DMI (Allen, 2000), or alter ruminal FA biohydrogenation (**BH**; Beam et al., 2000). Specifically, unsaturated FA often result in a greater decrease in DMI compared with saturated fat sources (e.g., Relling and Reynolds, 2007) and are associated with ruminal production of BH intermediates that induce milk fat depression (**MFD**; Harvatine et al., 2009).

Formation of calcium salts of unsaturated FA was devised over 30 yr ago to minimize the negative effects of PUFA on ruminal digestion of fiber (Jenkins and Palmquist, 1984; Palmquist, 1991). Most Ca-salt FA supplements available in the United States are derived from palm FA distillate, a by-product of palm oil processing, and contain a balance of saturated and unsaturated FA (approximately 50% C16:0 and 42% unsaturated FA). Importantly, Ca salt protection of PUFA from BH is incomplete (Wu et al., 1991), and the increased formation of BH intermediates such as *trans*-10 C18:1 indicates that PUFA negatively affect ruminal bacterial populations and BH pathways (Maia et al., 2007).

The FA profile of dietary fat has an effect on milk fat concentration and yield. Kadegowda et al. (2008) observed that abomasal infusion of a mix of short- and medium-chain SFA increased milk fat concentration and yield, whereas long-chain FA supplements had no effect compared with no fat infusion. Similarly, high-palmitic acid (**PA**) supplements were reported to increase milk fat yield (Steele and Moore, 1968; Enjalbert et al., 2000; Mosley et al., 2007). Specifically, Mosley et al. (2007) reported a linear increase in milk fat yield and C16:0 incorporation into milk fat when diet C16:0 concentration was increased from 1.8 to 5.2% of DM.

Received August 6, 2013.

Accepted February 16, 2014.

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Furthermore, higher mammary extraction efficiency of C16:0 compared with C18:0 was observed during duodenal infusions of C16:0 and C18:0 FA (Enjalbert et al., 1998, 2000). Lastly, C18:0 supplements have also been observed to increase milk fat yield in some instances, although the response is smaller compared with C16:0 supplementation (Steele and Moore, 1968; Enjalbert et al., 2000; Rico et al., 2014).

Within a herd, the physiology and metabolism of individual cows is very diverse and may interact with some dietary treatments. For example, cows with higher milk yields have greater energy demands, resulting in changes in DMI, ruminal pool size, ruminal passage rates, and microbial fermentation (e.g., Kammer et al. 2012). Harvatine and Allen (2006b) reported a reduction in milk fat in high- but not in low-producing cows when increasing diet unsaturated FA, presumably due to an interaction of the rumen environment of higher-production cows and unsaturated FA. Although not well explored, other differences in partitioning of absorbed fat between milk and body reserves are also possible.

The objective of the current experiment was to characterize the effect of a high-PA supplement on intake, milk and milk component yields, and total-tract digestibility in high- and low-producing dairy cows. The PA supplement was compared with a no-fat control and the well-investigated Ca salt of palm FA. The hypothesis was that a high-PA supplement would be ruminally inert and highly digestible, similar to previous reports of other saturated FFA supplements (Harvatine and Allen, 2006b,c). Additionally, high-producing cows were expected to increase milk yield, whereas low-producing cows were expected to increase milk fat yield when fed the high-PA supplement.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was conducted from May to July 2010 in a tie-stall barn located at the Pennsylvania State University Dairy Research and Teaching Center (University Park). All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Twelve high-milk yield cows (pretrial milk yield = 48.3 ± 7.2 kg/d; mean $\pm$ SD) and 12 low-milk yield cows (pretrial milk yield = 33.8 ± 3.7 kg milk/d; mean $\pm$ SD) were blocked by production level at the beginning of the study in a replicated 3×3 Latin square design with 21-d periods. All cows were multiparous Holsteins (688 ± 76 kg of BW) and DIM was similar between production blocks [211 ± 68 and 196 ± 43 DIM (mean $\pm$ SD) for high- and low-production cows, respectively].

Milk fat concentration differed between the production blocks when fed the control diet (3.14 and 3.86% milk fat in high- and low-production cows, respectively), but this was not a selection criterion. Three of the cows in the low- and 3 in the high-production block were ruminally cannulated. Treatments were (1) control (no supplemental fat), (2) high-palmitic FFA (PA) supplement (BergaFat F-100; Berg + Schmidt GmbH & Co., Hamburg, Germany), and (3) Ca salts of palm FA (**Ca-FA**) supplement (Megalac; Church & Dwight Co. Inc., Princeton, NJ). The PA supplement contained 84.8% C16:0 and 8.3% C18:1 and the Ca-FA supplement contained 47.4% C16:0, 35.9% C18:1, and 8.4% C18:2 (% of total FA; Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7341>). Both fat supplements were fed to provide approximately 2% FA in the diet on a DM basis. All cows received bST (Posilac; Elanco Animal Health, Greenfield, IN) every 14 d. Milk yield and DMI are known to slightly increase and then decrease during the bST cycle. The 14-d cycle and 21-d periods resulted in supplementation occurring on different days in each period. Little reason exists to expect an interaction between the small changes during the bST cycle and the treatments and changes during the cycle were accounted for by the period effect in the model.

Milk Sampling and Analysis

Cows were milked twice daily at 0500 and 1700 h and milk yield determined by an integrated milk meter (Afimilk; SAE Afikim, Kibbutz Afikim, Israel). Milk samples were taken at 6 consecutive milkings on d 19 to 21 of each period. A subsample from each milking was stored at 4°C with liquid preservative (Bronolab-WII; Dairy One Lab, State College, PA) until analyzed for fat (Filter B), protein, and lactose by infrared spectroscopy [Fossomatic 4000, MilkoScan, and Fossomatic 400 (Foss Electric A/S, Hillerød, Denmark); AOAC International (2000) method 972.160 (Dairy One laboratory, State College, PA)]. A second subsample of each milking on d 21 was stored at -20°C without preservative until analyzed for FA profile as described by Rico and Harvatine (2013). Briefly, lipids were extracted with hexane:isopropanol, base transmethylated with sodium methoxide, and quantified by gas chromatography with a flame ionization detector (**GC-FID**; Agilent 6890A; Agilent Technologies Inc., Santa Clara, CA) and a capillary column [SP-2560; 100 m $\times$ 0.25 mm (i.d.) with 0.2- μ m film thickness; Supelco Inc., Bellefonte, PA]. Milk FA are reported as nonesterified FA calculated as described by Rico and Harvatine (2013).

Energy-corrected milk (kg/d) was calculated as $(0.327 \times \text{milk kg/d}) + (12.95 \times \text{fat kg/d}) + (7.65 \times$

protein kg/d) (Dairy Records Management Systems, 2011).

Total-Tract Digestion Sample Collection and Analysis

Cows were fed once daily (0900 h) at 110% of expected intake based on the previous day intake. Diet ingredients (250 g) and individual orts (12.5%) were sampled daily from d 19 to 21 and composited by period. Six fecal samples were collected from d 19 to 21 of each period to represent every 4 h of the day. All feed, orts, and fecal samples were lyophilized (Ultra 35-XL; Virtis Co. Inc., Gardiner, NY) and ground in a Wiley mill (A. H. Thomas Co., Philadelphia, PA) using a 1-mm screen. Feeds were analyzed for DM, CP, NDF, indigestible NDF, ADF, starch, and FA concentration and profile. Orts and fecal samples were composited by period, lyophilized, and analyzed for DM, NDF, indigestible NDF, and FA concentration and profile. Nitrogen concentration was determined on a Thermo-Fisher EA 1110 CHNS elemental analyzer (Thermo Fisher Scientific Inc., Waltham, MA) and multiplied by 6.25 to obtain CP. Determination of NDF and ADF was carried out using the Ankom A200 filter bag technique (Ankom Technology, Macedon, NY) according to Van Soest et al. (1991) using a heat-stable amylase and sodium sulfite. Indigestible NDF (**iNDF**) was determined similarly to Huhtanen et al. (1994). Briefly, iNDF was determined as the NDF residue after 288 h in situ incubation in 25- μ m-pore-size filter bags (Ankom Technology) enclosed in a mesh bag in a lactating cow fed a diet similar to the experimental control diet. Bags were anchored with weights to be positioned centrally in the rumen. Following removal from the rumen, bags were rinsed manually in cold water until outflow was clear, and then analyzed for NDF content. Starch concentration was determined by an enzymatic method (Karkalas, 1985) after gelatinization with sodium hydroxide followed by glucose, determined using the glucose oxidase method (PGO enzyme product no. P7119; Sigma Chemical Co., St. Louis, MO; Allen and Ying, 2012). Feed particle size was determined on previously frozen TMR samples from period 1 and 2 using the Penn State Particle Size Separator (Nasco, Fort Atkinson, WI) with a 19-, 8-, and 4-mm screen (samples from period 3 were dried before storage and thus not analyzed).

Feed, orts, and fecal FA concentration and profile were determined by GC-FID after direct methylation (Sukhija and Palmquist, 1988). Briefly, FA were acid methylated and extracted in hexane. Methyl esters were quantified by GC-FID as described above with a modified temperature program (70°C for 4 min, 8°C/

min to 110°C, 5°C/min to 170°C for 10 min, and 4°C/min to 215°C for 23 min). Peaks were identified using pure methyl ester standards (GLC 60 and 780; Nu-Chek Prep Inc., Elysian, MN) and recoveries of individual FA determined using an equal weight reference standard (GLC 74; Nu-Chek Prep Inc.). Total, total 16-C, and total 18-C FA concentrations were estimated using C13:0 or C17:0 as internal standards (**ISTD**; Nu-Chek Prep Inc.) according to the following equations:

$$\text{Sample FA (\% of DM)} = [(\text{ISTD weight/ISTD concentration}) - \text{ISTD weight}] \times (\text{sample weight})^{-1};$$

$$\begin{aligned} 16\text{-C FA (\% of DM)} = & [(\text{ISTD weight} \times 16\text{-C FA} \\ & \text{concentration})/\text{ISTD concentration}] \\ & \times (\text{sample weight})^{-1}; \end{aligned}$$

$$\begin{aligned} 18\text{-C FA (\% of DM)} = & [(\text{ISTD weight} \times 18\text{-C} \\ & \text{FA concentration})/\text{ISTD concentration}] \\ & \times (\text{sample weight})^{-1}. \end{aligned}$$

Each sample was run in duplicate using C13:0 in the first run and C17:0 in the second to correct for endogenous levels of the standards. The endogenous concentrations of the standards were accounted for in determination of the ISTD concentration in the above equations.

Indigestible NDF was used as an internal marker for calculation of apparent total-tract DM, NDF, and FA digestibility. Offered and refused amounts and composition from d 19 to 21 were used to calculate nutrient intake. Daily flow of fecal DM was calculated as described by Huhtanen et al. (1994).

Ruminal pH was monitored in 6 ruminally cannulated cows using indwelling ruminal pH probes (Kahne Ltd., Auckland, New Zealand). Probes were programmed to record ruminal pH every 5 min during 3 separate 24-h periods from d 17 to 21 of each period (5 probes were rotated between cows over these days). Probes were calibrated daily (pH 4.0 and 7.0 at 39°C) before insertion and after removal. Data were discarded if probes were outside of ± 0.1 pH units from either calibration point at removal. Data were processed and analyzed using Igor Pro 6.2.2.2 (WaveMetrics Inc., Lake Oswego, OR) as described by Harvatine and Allen (2006a).

Statistical Analysis

Data were analyzed using the Fit Model procedure and the REML method of JMP (version 9.0.2; SAS

Institute Inc., Cary, NC). The model was $Y_{ijklm} = \mu + S_i + P_j + C_k(S_i) + T_l + B_m + T_l \times B_m + e_{ijklm}$, where Y_{ijklm} is the variable of interest, μ is the overall mean, S_i is the random effect of sequence ($i = 1$ to 6), P_j is the random effect of period ($j = 1$ to 3), $C_k(S_i)$ is the random effect of cow nested in sequence ($k = 1$ to 24), T_l is the fixed effect of treatment ($l = 1$ to 3), B_m is the fixed effect of production level block ($m = 1$ to 2), $T_l \times B_m$ is the interaction of treatment and production level block, and e_{ijklm} is the residual error. Additionally, the interaction of period and treatment was tested and was not significant ($P > 0.10$) and, therefore, removed from the model. All data are reported by production level because a significant treatment by production level interaction existed for key response variables. The preplanned contrasts were control versus PA and PA versus Ca-FA within each production level. These contrasts were designed to test the effect of PA compared with the no-fat control and a commonly fed and well investigated fat supplement (Ca-FA). Significant differences were declared at $P < 0.05$ for main effects and $P < 0.10$ for interactions, and tendency for a difference declared at $P < 0.10$ for main effects and $P < 0.15$ for interactions. Data were logarithmically transformed when appropriate and back-transformed data are reported. Data points with studentized residuals outside of ± 3.0 were considered outliers and excluded from analysis, but this rarely occurred for more than 1 value per variable.

RESULTS

The PA supplement contained 79% more PA than the calcium salts of palm FA at the expense of oleic and linoleic acid. Relative to the control diet, the PA supplement replaced 1.9% of dietary forage (Table 1). The control and PA diets contained 0.6% ground limestone to equalize calcium concentration. The control diet contained 3.8% FA and the PA and Ca-FA diets contained 5.6 and 5.7% FA, respectively. Diets contained similar concentrations of NDF, ADF, and CP (Table 1) but differed in concentration of total FA and, specifically, C16:0 and C18:1, as expected, based on the FA profile of the supplements tested (Table 1; Supplemental Table S2; <http://dx.doi.org/10.3168/jds.2013-7341>). The average particle size of the diets was 5.61 mm (7.1% >19 mm, 34.0% >8 mm, 21.2% >4 mm, and 37.8% <4 mm).

Milk Yield and Milk Composition

No treatment by production-level interactions existed for milk yield, ECM, fat yield, protein yield, and milk concentration of protein and lactose (Table 2). Milk

yield was not affected by treatment, but differed between production levels ($P < 0.05$; 42.1 ± 2.67 and 28.9 ± 2.50 kg/d for high- and low-production cows, respectively). Similarly, ECM was not affected by treatment and averaged 40.0 ± 2.50 kg/d in the high-producing cows and 30.3 ± 2.22 kg/d in the low-producing cows.

Milk fat yield was not affected by treatment and averaged 1.29 ± 0.10 kg/d in the high-producing cows and 1.09 ± 0.08 kg/d in the low-producing cows. However, a treatment by production level interaction for milk fat concentration ($P < 0.05$) was detected. Compared with PA, Ca-FA decreased milk fat concentration in the high-producing cows ($P < 0.01$; 3.22 and $2.86 \pm 0.23\%$, respectively); however, in the low-producing cows, no effect of treatment on milk fat concentration (average $3.88 \pm 0.2\%$) was detected. Milk lactose concentration was decreased by PA relative to the control by 0.11 and

Table 1. Ingredient and nutrient composition of experimental diets

Composition	Diet ¹		
	Control	PA	Ca-FA
Ingredient, % of DM			
Corn silage ²	40.5	39.7	40.5
Grain mix ³	37.2	36.1	35.7
Alfalfa haylage ⁴	13.8	14.0	13.7
Cottonseed hulls	3.4	3.3	3.4
Grass hay/straw mix	2.8	2.7	2.8
Sugar cane molasses	1.6	1.6	1.6
Ground limestone	0.6	0.6	—
Ca salts of FA	—	—	2.3
PA FA	—	1.9	—
Nutrient composition ⁵			
DM	52.8	53.1	52.9
NDF	31.5	30.9	31.1
Starch	26.0	25.4	25.6
CP	16.1	15.8	15.6
Total FA	3.8	5.6	5.7
C16:0	0.58	2.12	1.48
C18:1 (total) ⁶	0.85	0.98	1.52
C18:2n-6	1.68	1.67	1.81

¹Control = no supplemental fat; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

²Corn silage contained 38.3% DM (as fed), and 37% NDF, 33.8% starch, and 6.7% CP on a DM basis.

³Grain mix contained 30.6% canola meal, 30.3% ground corn, 18.6% cookie meal, 11.4% roasted soybeans, 8.1% mineral premix, and 1.0% Optigen [NPN source (278% CP, DM basis); Alltech Inc., Lexington, KY]. Mineral mix contained (as-fed basis) 45.8% dried corn distillers grains with solubles; 35.8% limestone (38% Ca); 8.3% magnesium oxide (54% Mg); 6.4% salt; 1.73% vitamin ADE premix; 1.09% selenium premix (0.06% selenium); and 0.88% trace mineral mix. Composition (DM basis): 11% CP, 18% NDF, 5.2% fat, 14.9% Ca, 0.35% P, 4.58% Mg, 0.41% K, 0.31% S, 357 mg of Cu/kg, 1,085 mg of Zn/kg, 181 mg of Fe/kg, 6.67 mg of Se/kg, 276,925 IU of vitamin A/kg, 69,120 IU of vitamin D/kg, and 2,081 IU of vitamin E/kg.

⁴Alfalfa haylage contained 47.7% DM (as fed), and 45.5% NDF, 1.3% starch, and 18.5% CP on a DM basis.

⁵Concentrations of all nutrients except DM are expressed on a 105°C DM basis, unless otherwise stated.

⁶Sum of *cis*-9 C18:1 and *cis*-11 C18:1.

Table 2. Effects of palmitic acid supplementation on milk production and composition in high- and low-producing cows

Item	Block ¹	Treatment LSM ²			SEM	Contrast		P-value ³		
		Control	PA	Ca-FA		Con vs. PA	PA vs. Ca-FA	Trt	Block	Trt × block
Milk yield, kg/d										
Milk	High	41.5	42.0	42.7	2.7	0.37	0.80	0.70	<0.001	0.83
	Low	28.8	29.0	29.0	2.5	0.79	0.99			
ECM	High	39.7	40.5	40.0	2.5	0.59	0.73	0.88	<0.01	0.91
	Low	30.4	30.4	30.2	2.2	0.97	0.82			
Fat	High	1.28	1.33	1.26	0.10	0.51	0.34	0.69	0.11	0.81
	Low	1.09	1.10	1.09	0.08	0.91	0.88			
Protein	High	1.30	1.33	1.33	0.09	0.58	0.92	0.93	<0.001	0.72
	Low	0.93	0.93	0.92	0.08	0.93	0.72			
Lactose	High	1.92	1.89	1.95	0.13	0.62	0.31	0.68	<0.001	0.79
	Low	1.31	1.30	1.31	0.13	0.93	0.88			
Milk composition, %										
Fat	High	3.14	3.22	2.86	0.23	0.43	<0.01	<0.01	<0.01	0.04
	Low	3.86	3.92	3.86	0.20	0.39	0.42			
Protein	High	3.14	3.17	3.13	0.06	0.54	0.25	0.12	0.94	0.26
	Low	3.19	3.14	3.11	0.04	0.10	0.34			
Lactose	High	4.61	4.50	4.57	0.09	0.02	0.09	0.01	0.67	0.71
	Low	4.56	4.48	4.52	0.08	0.04	0.28			

¹Level of production: high = high-producing cows (>40 milk kg/d) and low = low-producing cows (<38 milk kg/d).

²Control (Con) = no supplemental FA; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

³Trt = treatment effect; block = effect of level of production; Trt × block = interaction of treatment and level of production.

0.08 percentage units in the high- and low-producing cows, respectively ($P < 0.05$), but lactose yield was not affected by treatment. No effect of treatment on milk protein concentration or yield was detected.

Milk FA by Source

A tendency existed for a treatment by production level interaction for the concentration of milk FA less than 16 C (de novo synthesized FA; $P = 0.13$; Figure 1a). In high-producing cows, PA decreased milk de novo FA concentration relative to the control by 11% ($P < 0.01$), and Ca-FA decreased de novo FA by 11% relative to PA ($P < 0.01$). However, in the low-producing cows, PA decreased de novo FA concentration by 13% relative to the control ($P < 0.001$), but was not different from Ca-FA. No treatment by production level interaction was detected for yield of milk de novo FA (Table 3). In the high-producing cows, Ca-FA tended to decrease de novo FA yield by 11% compared with PA ($P = 0.09$), but PA was not different from the control. Similar to the concentration of de novo FA, PA decreased the yield of de novo FA by 12% ($P < 0.05$) relative to the control but was not different from Ca-FA in the low-producing cows.

An effect of treatment, but no treatment by production level interaction existed for milk fat concentration and yield of 16-C FA (Figure 1b; Table 3). The PA supplement increased milk fat concentration and yield of 16-C FA by more than 25% relative to the control and more than 16% relative to Ca-FA ($P < 0.001$).

A treatment by production level interaction existed for the concentration of 18-C FA (preformed FA; $P = 0.07$; Supplemental Table S3; <http://dx.doi.org/10.3168/jds.2013-7341>; Figure 1c). The PA supplement reduced the 18-C FA concentration relative to the control by 12% in the high-producing cows and by 10% in the low-producing cows ($P < 0.001$). The PA supplement also reduced preformed FA concentration relative to Ca-FA by 19% in the high-producing cows and 13% in low-producing cows ($P < 0.001$). No treatment by production level interaction was detected for the yield of milk 18-C FA (preformed FA; Table 3). Compared with the control, PA tended to reduce preformed FA yield by more than 10% at both production levels ($P < 0.1$). However, compared with Ca-FA, PA reduced the yield of 18-C FA by more than 13% at both levels ($P < 0.05$; Table 3). The concentrations and yields of individual milk FA generally followed a similar pattern to their source as discussed above (Supplemental Tables S3 and S4; <http://dx.doi.org/10.3168/jds.2013-7341>).

Milk trans FA and Desaturase Indices

A treatment by production level interaction ($P < 0.05$) was detected for the yields of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA (Table 3). Milk fat yields of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA were not different between the control and PA at either production level ($P > 0.24$). However, in high-producing cows, Ca-FA increased the yields of *trans*-10 C18:1 and *trans*-

10,*cis*-12 CLA compared with PA by over 230% ($P < 0.01$). The milk fat concentrations of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA followed a similar pattern (Supplemental Table S3; <http://dx.doi.org/10.3168/jds.2013-7341>).

The PA supplement increased the concentration of saturated FA by 4 and 3% relative to the control and by 12 and 6% relative to Ca-FA in the high- and low-production levels, respectively ($P < 0.01$; Supplemental Table S3; <http://dx.doi.org/10.3168/jds.2013-7341>). The C14, C16, and C18 desaturase indices were not affected by treatment, except for a 16% increase in the C16 desaturase index in PA relative to the control in the high-producing cows ($P < 0.05$; Supplemental Table S3).

Nutrient Intake and Apparent Total-Tract Digestibility

No treatment by production level interaction was detected for DM or NDF intake (Table 4), although high-producing cows had higher DMI compared with the low-producing cows, as expected ($P < 0.01$). Intake was decreased by PA compared with the control (1.9 and 2.2 kg/d in high- and low-producing cows, respectively; $P < 0.01$), but was not different from Ca-FA. Intake of NDF followed a similar pattern.

A treatment by production level interaction existed for DM and NDF digestibility ($P < 0.05$; Table 4). In high-producing cows, DM digestibility was not different between treatments and averaged $61.2 \pm 0.5\%$. In the low-producing cows, PA increased DM digestibility relative to Ca-FA (63.8 vs. $61.7 \pm 0.48\%$, respectively; $P < 0.001$), but was not different from the control. No effect of treatment on NDF digestibility was detected in high-producing cows; however, in low-producing cows, PA tended to decrease NDF digestibility by 2.5 percentage units compared with Ca-FA ($P = 0.07$).

A treatment by production level interaction existed for the amount of NDF digested per day ($P < 0.05$; Table 4), but no interaction existed for amount of DM digested. In high-producing cows, PA tended to decrease digested DM relative to the control ($P = 0.08$). However, in low-producing cows, PA decreased DM digested relative to the control by 6.8%, but was marginally higher than Ca-FA (0.2 kg/d; $P < 0.05$). No effect of treatment was detected on NDF digested in high-producing cows, but PA decreased the NDF digested by 0.4 kg/d in low-producing cows ($P < 0.001$).

Feed Efficiency

Feed efficiency was not different between PA and Ca-FA at either production level, but relative to the control, PA increased feed efficiency by 8.5% in high-

Figure 1. Effect of a high-palmitic acid (PA) supplement on milk FA profile compared with a no-fat supplement (control) and calcium salts of palm FA (Ca-FA). The concentration of de novo synthesized (<16-C; panel A), total 16-C (panel B), and total 18-C (panel C) FA in high- and low-producing cows is shown. High = high-producing cows (mean milk yield = 42.1 kg/d); low = low-producing cows (mean milk yield = 28.9 kg/d). Preplanned contrasts tested the difference between control and PA (1 = $P < 0.001$, 1 = $P < 0.01$) and PA and Ca-FA (2 = $P < 0.001$, 2 = $P < 0.01$). Main-effect P -values for treatment, production block, and the interaction of treatment and block were $P < 0.001$, $P = 0.67$, and $P = 0.13$ for de novo; $P < 0.001$, $P = 0.21$, and $P = 0.24$ for total 16-C FA; and $P < 0.01$, $P = 0.19$, and $P = 0.07$ for total 18-C FA, respectively (SEM = 0.8, 1.13, and 1.11 for de novo, total 16-C FA, and total 18-C FA, respectively).

producing cows ($P < 0.05$; ECM/DMI) and tended to increase feed efficiency by 7.6% in low-producing cows ($P = 0.07$; Table 4).

Table 3. Effects of palmitic acid supplementation on yield of select milk FA in high- and low-producing cows

FA, g/d	Block ¹	Treatment LSM ²				Contrast		P-value ³		
		Control	PA	Ca-FA	SEM	Con vs. PA	PA vs. Ca-FA	Trt	Block	Trt × block
<i>trans</i> -10 C18:1	High	6.9	9.7	22.4	2.0	0.24	<0.001	<0.001	<0.001	<0.001
	Low	4.8	5.1	5.7	1.6	0.87	0.72			
<i>trans</i> -11 C18:1	High	14.6	11.7	14.2	1.4	0.06	0.09	<0.01	0.15	0.98
	Low	12.5	10.0	12.2	1.0	0.02	0.07			
<i>cis</i> -9, <i>trans</i> -11 CLA	High	8.6	7.5	10.3	1.1	0.20	0.01	<0.01	<0.001	0.85
	Low	6.7	5.3	6.6	1.1	0.04	0.33			
<i>trans</i> -10, <i>cis</i> -12 CLA	High	0.06	0.07	0.16	0.02	0.74	<0.01	0.02	<0.01	0.02
	Low	0.05	0.05	0.05	0.01	0.97	0.96			
Σ <16 C ⁴	High	293	272	241	27	0.27	0.09	<0.01	0.22	0.57
	Low	255	220	214	21	0.02	0.69			
Σ 16 C ⁴	High	351	468	372	37	<0.001	<0.001	<0.001	0.13	0.41
	Low	293	378	321	30	<0.001	<0.01			
Σ 18 C ⁴	High	461	405	476	30	0.05	0.01	<0.01	0.08	0.85
	Low	399	359	411	23	0.07	0.02			

¹Level of production: high = high-producing cows (>40 milk kg/d) and low = low-producing cows (<38 milk kg/d).

²Control (Con) = no supplemental FA; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

³Trt = treatment effect; block = effect of level of production; Trt × block = interaction of treatment and level of production.

⁴FA <16 C originate from de novo synthesis in the mammary gland; 18-C FA originate from extraction from plasma, and 16-C FA originate from both sources.

Ruminal pH

Rumen pH was measured on a subset of cows (n = 3 high-producing and n = 3 low-producing cows) to characterize the diets, although the power of statistical tests was limited by the sample size (Table 5; Supple-

mental Table S5; <http://dx.doi.org/10.3168/jds.2013-7341>). No effect of treatment, level, or treatment by production level interaction on mean pH (average = 5.97 ± 0.07) was detected. A treatment by production level interaction was detected for average pH range. The average pH range was higher in PA compared with

Table 4. Effects of palmitic acid supplementation on apparent total-tract digestion of DM and NDF in high- and low-producing cows

		Treatment LSM ²				Contrast		P-value ³		
Item	Block ¹	Control	PA	Ca-FA	SEM	Con vs. PA	PA vs. Ca-FA	Trt	Block	Trt × block
DM										
Intake, ⁴ kg/d	High	28.3	26.4	26.2	1.5	0.02	0.69	<0.01	<0.01	0.78
	Low	25.3	23.0	23.4	1.5	<0.01	0.53			
Digested, kg/d	High	16.1	15.3	15.0	0.9	0.07	0.49	<0.001	<0.01	0.96
	Low	14.7	13.7	13.5	0.9	0.02	<0.001			
Digestibility, %	High	61.1	61.1	61.3	0.5	0.99	0.69	0.08	<0.001	0.02
	Low	63.6	63.8	61.7	0.5	0.81	<0.001			
NDF										
Intake, kg/d	High	8.0	7.6	7.5	0.5	0.04	0.80	<0.001	<0.01	0.68
	Low	7.2	6.5	6.5	0.5	<0.01	0.65			
Digested, kg/d	High	2.6	2.6	2.5	0.4	0.86	0.81	0.06	0.23	0.03
	Low	2.7	2.3	2.4	0.4	<0.001	0.36			
Digestibility, %	High	31.2	33.4	33.4	2.7	0.14	0.95	0.84	<0.01	<0.05
	Low	37.0	34.5	35.6	2.7	0.07	0.38			
Feed efficiency ⁵	High	1.41	1.53	1.51	0.08	0.04	0.76	0.03	0.01	0.82
	Low	1.20	1.29	1.25	0.08	0.07	0.46			

¹Level of production: high = high-producing cows (>40 milk kg/d) and low = low-producing cows (<38 milk kg/d).

²Control (Con) = no supplemental FA; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

³Trt = treatment effect; block = effect of level of production; Trt × block = interaction of treatment and level of production.

⁴DMI is on a 55°C basis.

⁵Feed efficiency = ECM/DMI; ECM (kg/d) was calculated as $(0.327 \times \text{milk kg/d}) + (12.95 \times \text{fat kg/d}) + (7.65 \times \text{protein kg/d})$ (Dairy Records Management Systems, 2011).

Table 5. Effects of palmitic acid supplementation on ruminal pH in high- and low-producing cows (n = 6 per treatment)

Item	Block ¹	Treatment LSM ²			SEM	Contrast		P-value ³		
		Control	PA	Ca-FA		Con vs. PA	PA vs. Ca-FA	Trt	Block	Trt × block
Average pH	High	5.95	5.94	5.86	0.07	0.88	0.36	0.92	0.20	0.40
	Low	6.01	5.99	6.05	0.07	0.82	0.44			
pH range	High	1.62	2.01	1.84	0.15	<0.01	0.08	<0.01	0.08	0.03
	Low	1.39	1.68	1.22	0.15	0.01	<0.01			
Time below pH 5.6, h/d	High	4.82	5.15	6.34	1.36	0.75	0.28	0.33	0.16	0.05
	Low	2.96	4.57	1.31	1.36	0.16	0.02			

¹Level of production: high = high-producing cows (>40 milk kg/d) and low = low-producing cows (<38 milk kg/d).

²Control (Con) = no supplemental FA; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

³Trt = treatment effect; block = effect of level of production; Trt × block = interaction of treatment and level of production.

the control in high- and low-producing cows ($P < 0.01$) and the pH range was higher in PA than Ca-FA in low-producing cows ($P < 0.01$).

A treatment by production level interaction was detected for time below pH 5.6. The time below pH 5.6 was not affected by treatment in high-producing cows. However, in low-producing cows, PA increased time under pH 5.6 by 3.3 h/d ($P < 0.05$). Time under pH 5.8 followed a similar pattern (Supplemental Table S5; <http://dx.doi.org/10.3168/jds.2013-7341>).

FA Intake and Apparent Total-Tract Digestibility

No treatment by production level interaction existed for intake of total, 16-C, and 18-C FA, but all were

higher in high- than in low-producing cows ($P < 0.001$; Table 6). At both production levels, PA increased digested total FA by more than 250 g/d and 16-C FA by more than 240 g/d compared with the control. The PA supplement also increased digested 16-C FA by more than 90 g/d compared with Ca-FA ($P < 0.01$), whereas it decreased digested 18-C FA relative to Ca-FA by more than 90 g/d ($P < 0.001$) at both production levels.

No treatment by production level interaction for total FA digestibility was detected, but PA increased total FA digestibility by 8.6% relative to the control in low-producing ($P < 0.05$), but not in high-producing cows. Total FA digestibility was not different between PA and Ca-FA at either production level. A treatment

Table 6. Effects of palmitic acid supplementation on total-tract digestion of FA in high- and low-producing cows

		Treatment LSM ²				Contrast		P-value ³		
Item	Block ¹	Control	PA	Ca-FA	SEM	Con vs. PA	PA vs. Ca-FA	Trt	Block	Trt × block
Total FA										
Intake, kg/d	High	0.99	1.37	1.37	0.08	<0.001	0.97	<0.001	<0.01	0.87
	Low	0.86	1.17	1.19	0.08	<0.001	0.49			
Absorbed, kg/d	High	0.54	0.81	0.85	0.07	<0.001	0.35	<0.001	0.08	0.37
	Low	0.51	0.76	0.74	0.06	<0.001	0.61			
Digestibility, %	High	55.3	58.9	61.9	2.41	0.10	0.19	<0.01	0.22	0.20
	Low	59.3	64.3	62.1	2.15	0.02	0.24			
16-C FA										
Intake, kg/d	High	0.15	0.53	0.36	0.03	<0.001	<0.001	<0.001	<0.001	0.01
	Low	0.13	0.46	0.32	0.02	<0.001	<0.001			
Absorbed, kg/d	High	0.09	0.34	0.25	0.02	<0.001	<0.001	<0.001	0.06	0.31
	Low	0.08	0.32	0.21	0.02	<0.001	<0.001			
Digestibility, %	High	57.6	63.5	67.4	2.33	0.01	0.08	<0.001	0.21	0.05
	Low	62.0	69.5	66.5	2.13	<0.001	0.11			
18-C FA										
Intake, kg/d	High	0.75	0.75	0.91	0.05	0.80	<0.001	<0.001	<0.01	0.72
	Low	0.66	0.63	0.80	0.05	0.27	<0.001			
Absorbed, kg/d	High	0.44	0.44	0.58	0.03	0.94	<0.001	<0.001	0.09	0.33
	Low	0.42	0.41	0.50	0.03	0.79	<0.001			
Digestibility, %	High	59.1	59.3	63.2	2.63	0.92	0.12	0.54	0.21	0.21
	Low	63.7	65.0	63.3	2.42	0.57	0.42			

¹Level of production: high = high-producing cows (>40 milk kg/d) and low = low-producing cows (<38 milk kg/d).

²Control (Con) = no supplemental FA; PA = high-palmitic FFA supplement; Ca-FA = calcium salts of palm FA.

³Trt = treatment effect; block = effect of level of production; Trt × block = interaction of treatment and level of production.

by production level interaction for 16-C FA digestibility ($P = 0.05$) was detected. Relative to the control, PA increased digestibility of 16-C FA by more than 10% at both levels ($P < 0.05$). However, PA tended to decrease 16-C FA digestibility relative to Ca-FA by 5.8% in the high-producing cows ($P = 0.08$), but not in the low-producing cows. Digestibility of 18-C FA was not affected by treatment.

DISCUSSION

The effect of dietary supplementation of PA was assessed by comparison to a control diet with no supplemental fat and to a diet supplemented with Ca-FA of palm FA, which is a well-investigated and commonly used supplement in dairy cow rations. The PA supplement was distilled FFA concentrated in C16:0, designed to minimize negative effects on microbial fermentation. In contrast, the Ca-FA supplement was a source of both saturated (mainly C16:0) and unsaturated (mainly C18:1 and C18:2) FA complexed with a calcium ion to slow down rumen availability. The fat supplement feeding rate was between 440 and 500 g/d in the low- and high-producing cows, respectively, and is comparable to other studies using similar supplements (e.g., Palmquist, 1991; Enjalbert et al., 2000; Warntjes et al., 2008). Cow milk production level was used as a blocking factor to investigate a treatment interaction with metabolic demand. Although production levels were similar in DIM, they differed considerably in average milk production (28.9 and 42.1 kg/d) and milk fat concentration, similarly to the study of Harvatine and Allen (2006b).

Long-chain SFA supplements normally have little effect on DMI, whereas unsaturated FA supplements tend to reduce DMI (Allen, 2000; Rabiee et al., 2012). For example, a saturated FA supplement fed at 3% of ration DM had no effect on DMI compared with both a low-fat control and calcium salts of palm FA (Weiss et al., 2011). Additionally, when saturated and unsaturated FA supplements were directly compared, unsaturated FA decreased DMI (Bremmer et al., 1998; Harvatine and Allen, 2006a; Relling and Reynolds, 2007). However, the effect of highly enriched PA supplements on DMI has not been extensively investigated and reported results are inconsistent. An enriched C16:0 supplement similar to that used in the current experiment fed at a similar rate increased DMI in Mosley et al. (2007), but had no effect during summer in Warntjes et al. (2008) or during cooler conditions in Piantoni et al. (2013). In the current experiment, the reduction in DMI by PA supplementation was unexpected, as Harvatine and Allen (2006a) observed no difference between a saturated fat supplement that included both C16:0 and C18:0 and

a no-supplement control. The observed response could be related to higher dietary energy density compared with control or to the hypophagic effects of gut peptides, such as glucagon-like peptide 1 and cholecystokinin (CCK), which are increased by dietary fats (Relling and Reynolds, 2007; Bradford et al., 2008). Although not measured in the present experiment, glucagon-like peptide 1 and CCK were increased by a hydrogenated FFA supplement (Relling and Reynolds, 2007) and CCK was increased by a high-purity 16:0 supplement relative to no fat supplementation (Piantoni et al., 2013). Furthermore, although PA was more saturated than the Ca-FA supplement, we observed no differences in feeding behavior measurements in a subset of 9 cows (i.e., number of meals, meal length, and time interval between meals; data not shown).

Unsaturated FA can have toxic effects on ruminal bacteria and decrease ruminal digestibility of fiber (Jenkins, 1993; Maia et al., 2007). Overall, the PA supplement had only minor effects on total-tract DM and NDF digestibility, demonstrating little or no effect on ruminal fiber digestibility. Similarly, previous direct comparisons of a saturated prilled FA supplement to Ca salts of palm FA fed at 3.0 to 3.5% of diet DM showed no effect on DM or NDF digestibility in low-producing cows (18 to 25 kg of milk/d; Grummer, 1988; Schauuff and Clark, 1989; Palmquist, 1991) or in high-producing cows (42 kg of milk/d; Weiss et al., 2011). In contrast to these results, feeding a PA-enriched supplement tended to increase and increased DM and NDF total-tract digestibility (Warntjes et al., 2008; Piantoni et al., 2013) in high-production cows (37 and 45 kg of milk/d, respectively).

Digestibility of FA in the current experiment was similar to previous reports by Grummer (1988), but modestly lower than reported in similar studies in low- (Palmquist, 1991) and high-producing cows (Harvatine and Allen, 2006c; Warntjes et al., 2008; Weiss et al., 2011). Dry matter and NDF total-tract digestibility values are similar to those previously reported in high-producing cows (e.g., Harvatine and Allen, 2006c; Tekippe et al. 2013), although they are moderately lower than much of the literature (Huhtanen et al. 2009). Lee and Hristov (2013) reported decreased digestibility with a similar iNDF marker compared with total fecal collection. We do not have reasons to expect a systematic bias between treatments and the lower values may be due to diet or methodology factors. Regardless of level of production, PA had positive effects on total FA digestibility, which was mainly explained by an increase in 16-C FA digestibility. Although it is difficult to determine the specific digestibility of the PA supplement, it was more digestible than FA in the basal diet. Digestibility values were higher for 16-C compared

with 18-C FA at both production levels; however, the difference was rather small (2.2 percentage units). In agreement, higher digestibility values have been previously reported for 16-C compared with 18-C FA (Elliott et al., 1995; Harvatine and Allen, 2006c). In contrast, total FA digestibility was decreased by a highly enriched (99%) C16:0 supplement compared with a no-fat supplement control mainly due to a reduction in 16-C FA digestibility (Piantoni et al., 2013).

The Latin square design with 21-d periods used in the current study allowed for the evaluation of the short-term milk production response to fat supplements, which provides mechanistic insight into the primary response to the supplement. Previous studies have shown increased or a tendency for increased milk yield when feeding high-PA supplements to low- and moderate-milk yield cows (Enjalbert et al., 1997; Mosley et al., 2007; Warntjes et al., 2008). In high-producing cows, PA increased milk and FCM yields, compared with a Ca-FA supplement (our unpublished data). Milk yield was not affected by treatment in the current experiment, and the explanation for these differences is not clear. Although milk yield and ECM were not increased by PA supplementation, the observed reduction in DMI resulted in higher feed efficiency compared with no fat supplementation. Longer-term feeding may result in secondary adaptations in intake, milk production, and energy balance, which should be investigated in future studies.

In high-producing cows, milk fat concentration was low compared with the typical average for Holstein herds. Importantly, average milk fat across all cows was 3.5%, which would not be considered MFD, and is close to typical values observed for Holstein cows during summer months (Bailey et al., 2005). Cows were blocked based on milk yield, and the difference in milk fat highlights the physiological and metabolic differences between the high- and low-production groups. Despite efficient absorption of FA in the PA treatment, it did not increase milk fat concentration or yield compared with the control. Early studies in low-producing cows fed a high-purity PA supplement (85 to 96% purity) at 3.9 to 4.6% of DM reported a 13 to 26% increase in milk fat concentration and a 21% increase in milk fat yield relative to a low-fat control (Steele and Moore, 1968; Steele, 1969). More recently, a similar highly enriched PA supplement (87% C16:0) fed at 1.8% of DM increased milk fat concentration by 14% and yield by 28% (Mosley et al., 2007), but had no effect in another experiment when fed at 1.7% of DM (Warntjes et al., 2008). Additionally, duodenal infusion of 500 g/d of a high-purity PA supplement (99% C16:0) increased total milk FA concentration by 27% and yield by 30% relative to no-fat infusion (Enjalbert et al.,

2000). In the present experiment, the lack of a positive effect of PA on milk fat yield appears to be due to the reduced yield of de novo synthesized FA (21 and 35 g decrease in high- and low-producing cows, respectively) and preformed FA (40 and 56 g/d in high- and low-producing cows, respectively), as the 16-C FA yield was dramatically increased relative to the control (117 and 85 g/d in high- and low-producing cows, respectively).

Enrichment of milk fat with 16-C FA was expected, as increases of 37 to 57% have been reported with PA treatments (Steele and Moore, 1968; Noble et al., 1969). Similar to our results, Steele and Moore (1968) reported decreased yields of milk C10:0, C12:0, C14:0, and 18-C FA. However, other experiments that reported increased milk fat with PA supplementation did not have a decrease in yield of de novo synthesized FA. For example, Mosley et al. (2007) reported an almost 100% increase in C16:0 yield and no decrease in the yield of de novo synthesized FA. However, in the current experiment, the increase in C16:0 yield was offset by a decrease in FA from other sources.

Kinsella and Gross (1973) reported that PA was the main FA used for initiation of glycerolipid synthesis by acylation at the sn-1 position. In addition, Hansen and Knudsen (1987) reported that PA stimulated de novo FA synthesis and incorporation into triglycerides, whereas other FA (C18:0, C18:1, and C18:2) were neutral or had no effect in dispersed goat mammary epithelial cells. In contrast, in the present experiment, PA decreased de novo FA concentration and secretion, suggesting that de novo synthesis was partially inhibited by PA or that it competed with other FA for incorporation into triglycerides. Importantly, long-chain acyl-CoA can decrease acetyl-CoA carboxylase (ACC) activity by promoting enzyme depolymerization (Clarke and Clarke, 1982) and Wright et al. (2002) proposed that PA inhibits FA synthesis by allosteric interaction with FA synthase or ACC. Although not measured in the current experiment, it is possible that the observed reduction in milk de novo FA was related to changes in the activity of FA synthase or ACC. Interestingly, a difference in the extent of inhibition was observed for specific de novo synthesized FA in the current experiment (Figure 2). A larger decrease was observed in the medium-chain FA than the short-chain FA with PA supplementation, whereas Ca-FA resulted in a more consistent decrease in individual FA in both low-producing and the high-producing cows where diet-induced MFD was observed. It appears that the mechanism of inhibition of de novo FA synthesis by PA differs from the mechanism of diet-induced MFD, although little is known about the molecular mechanism of PA in the mammary gland. Importantly, the success of a PA supplement to increase milk fat yield depends on its

Figure 2. Inhibition of individual de novo synthesized FA by a high-palmitic acid (PA; panel A) and calcium salts of palm FA (Ca-FA; panel B) supplement compared with the control in high- and low-producing cows. Milk yield of the high-producing (high) block averaged 42.1 kg/d and the low-producing (low) block averaged 28.9 kg/d. Percentage inhibition is calculated as [(supplement – control)/control] $\times$ 100.

effect on de novo FA synthesis and on incorporation of other preformed FA into milk fat. Future work will be required to understand the role of PA in regulation of mammary lipogenesis.

A recent meta-analysis reported a small positive effect of Ca salts of palm FA on milk fat concentration and yield (Rabiee et al., 2012). In contrast, we observed a reduction in milk fat concentration by Ca-FA relative to PA in the high-producing cows but not the low-producing cows. Harvatine and Allen (2006b) similarly reported that calcium salts of a blend of FA higher in unsaturated FA than palm FA resulted in decreased milk fat yield in high- but not low-producing cows. Calcium salts were designed to minimize negative effects of FA on fiber digestibility, not to completely protect the FA from BH (Palmquist, 1991). The extent of the protection from BH can be affected by ruminal pH, FA chain length, and degree of unsaturation (Wu et al., 1991; Enjalbert et al., 1997). Decreased rumi-

nal pH elicits changes in ruminal bacteria populations expected to increase the production of BH intermediates in the altered pathway, such as *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA (Fuentes et al., 2009). However, in the present study, ruminal pH was minimally affected, suggesting that the observed increases in these FA in the high-producing cows were due to other factors, most likely an interaction of PUFA and increased rumen passage in high-intake cows. Even though the concentration of C18:2 in Ca-FA was only 8.3% of total FA, this translated into an increase of approximately 24% in the dietary concentration of unsaturated FA relative to the PA diet. Dry matter intake was higher in the high- than in the low-producing cows and, although not measured, it is reasonable to expect that ruminal passage rates were increased. The rumen dynamics of high- and low-intake cows are not completely understood, but the high passage rate is expected to modify the ruminal microbial population and increase ruminal outflow of BH intermediates before complete BH occurs. It is clear that these changes can result in production of intermediates of the alternate BH pathway, such as *trans*-10 C18:1 FA and *trans*-10,*cis*-12 CLA, which are associated with classical diet-induced MFD.

Finally, in the current experiment and others, feeding high-purity PA supplements results in a modest increase in the concentration of total SFA in milk fat and is associated with a dramatic increase in C16:0 concentration (Steele and Moore, 1968; Mosley et al., 2007). For decades, saturated fat intake has been considered a strong risk factor for human cardiovascular disease, as it has been associated with increased blood cholesterol concentration (Ulbricht and Southgate, 1991). However, recent meta-analyses have found that saturated fat intake does not increase the total to high-density lipoprotein cholesterol ratio, nor is it related to fatal or nonfatal cardiovascular disease events (Mensink et al., 2003; Siri-Tarino et al., 2010a). It has been suggested that the replacement of SFA with PUFA, but not with carbohydrates, can reduce the occurrence of cardiovascular disease (Siri-Tarino et al., 2010b). The effect of saturated fat in humans remains a controversial issue.

CONCLUSIONS

Short-term feeding of a PA supplementation increased feed efficiency and substantially affected the milk FA profile in high- and low-producing cows relative to no fat supplementation; however, the increase in milk fat reported by others was not observed due to decreased de novo and preformed FA incorporation into milk fat. The PA supplement increased the digestibility of dietary FA compared with the control, but appeared to have little effect on digestion of other diet components.

Additionally, the PA supplement posed less risk for diet-induced MFD compared with a more unsaturated calcium salt of palm FA in high-producing cows.

ACKNOWLEDGMENTS

The authors thank Mutian Niu, Andrew Clarke, Kaitlyn Proschold, Megan Deeds, Grace Takacs, and Whitney Rottman-Gredell for their technical assistance at Penn State University (University Park). In addition, we thank Walter Kennett (Kennstock Farms, Pottsville, PA), Al Ames (NutriLinx LLC, Hardwick, VT), and R. Adelman (Berg + Schmidt GmbH & Co., Hamburg, Germany) for helpful discussion and Berg + Schmidt GmbH & Co. for partial financial support of the study.

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