Short communication: Comparison of a palmitic acid-enriched triglyceride supplement and calcium salts of palm fatty acids supplement on production responses of dairy cows

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ABSTRACT

The objective of our study was to evaluate the effects of feeding a palmitic acid-enriched triglyceride supplement or a calcium salts of palm fatty acid (FA) supplement on nutrient digestibility and production responses of mid-lactation dairy cows. Fifteen Holstein cows (139 ± 39 d in milk) were randomly assigned to treatment sequence in a 3 × 3 Latin square design. Treatments were a control diet (CON; no fat supplement) and 1.5% of FA added either as a palmitic acid-enriched triglyceride supplement (PA-TG) or as calcium salts of palm FA supplement (Ca-FA). Fat-supplemented treatments did not affect dry matter intake (DMI) compared with CON, but Ca-FA reduced DMI compared with PA-TG. Compared with CON, fat-supplemented treatments increased 18-carbon FA digestibility by 2.0 percentage units but did not affect digestibility of total FA or 16-carbon FA. Compared with Ca-FA, PA-TG reduced total FA digestibility by 8.7 percentage units due to a decrease in 16-carbon FA digestibility (21.7 percentage units). Both fat supplements increased neutral detergent fiber (NDF) digestibility compared with CON (3.90 percentage units), and PA-TG tended to increase NDF digestibility by 1.60 percentage units compared with Ca-FA. Compared with CON, fat-supplemented treatments increased milk yield (1.05 kg/d), 3.5% fat-corrected milk yield (2.20 kg/d), and energy-corrected milk yield (1.80 kg/d). Also, PA-TG increased milk fat yield (50 g/d) and milk energy output (1.0 Mcal/d) and tended to increase milk fat content (0.07 percentage units) and energy-corrected milk yield (1.0 kg/d) compared with Ca-FA. Fat-supplemented treatments reduced the yield of de novo milk FA (23 g/d) and increased the yields of mixed (43 g/d) and preformed (52 g/d) milk FA compared with CON. The PA-TG treatment increased the yield of 16-carbon (66 g/d) milk FA compared with Ca-FA, whereas Ca-FA increased the

yield of preformed (60 g/d) milk FA. Fat-supplemented treatments increased intake of net energy for lactation by 1.80 Mcal/d, milk energy output by 1.30 Mcal/d, and energy in body reserves by 0.30 Mcal/d compared with CON. The Ca-FA treatment increased energy allocated to body reserves (0.60 Mcal/d), energy partitioning toward body reserves (1.20 percentage units), and body condition score change (0.06 units), and tended to increase body weight change (0.16 kg/d) and body condition score (0.08 units) compared with PA-TG. In conclusion, feeding a palmitic acid-enriched triglyceride supplement increased milk energy output due to increased yields of milk and milk fat, whereas feeding a calcium salts of palm FA supplement increased FA digestibility and energy partitioned to body reserves.

Key words: palmitic acid, triglyceride, fatty acid, production, calcium salts of palm fatty acid

Short Communication

Fat supplements are commonly added to dairy cow diets to increase dietary energy density, feed efficiency, and yields of milk and milk fat, and to improve energy balance (Palmquist, 1994; Rabiee et al., 2012). One of the most common sources of rumen-protected fat is calcium salts, conceived over 30 yr ago to minimize the negative effects of UFA on ruminal populations, especially on cellulolytic bacteria (Palmquist, 1991). Individual fatty acids (FA) can have different effects and considerable recent research has focused on palmitic acid (C16:0) supplementation. Palmitic acid has been reported to increase milk fat concentration and yield and the efficiency of milk production compared with non-fat-supplemented diets (Lock et al., 2013; de Souza et al., 2017) and with other fat supplements (Rico et al., 2014a,b). However, with the exception of Weiss et al. (2011), published studies in lactating dairy cows have evaluated the effect of palmitic acid-enriched free FA supplements rather than triglyceride (TG) supplements.

Changes in intake and nutrient digestibility due to supplemental fat may affect, positively or negatively,

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digestible energy available for milk production, body reserves, or both (Boerman et al., 2015a). Weiss et al. (2011) observed lower total FA digestibility for a palmitic acid-enriched TG supplement compared with a nonfat control diet when fed at 3% of diet DM. However, the decrease in FA digestibility was smaller than that previously reported in other studies that fed saturated TG supplements, in which total FA digestibility ranged from 33 to 62% (e.g., Pantoja et al., 1995; Elliott et al., 1999; Weiss and Wyatt, 2004). Although these results suggest that a TG supplement with a low proportion of C18:0 (and high proportions of C16:0 and C18:1) has moderate digestibility (Weiss et al., 2011), comparative data for feeding palmitic acid-enriched TG supplements to lactating dairy cows are not available. Importantly, considering that most dairy farms that use supplemental fat would include it in diets within a range of 0.5 to 2.0% of ration DM, determining the effects of a palmitic acid-enriched TG supplement within this range has important applications. Therefore, characterizing the effects of feeding a palmitic acid-enriched TG supplement under typical feeding conditions is of particular importance.

The objective of the current experiment was to characterize the effects of feeding a palmitic acid-enriched TG supplement on nutrient digestibility and production responses of mid-lactation dairy cows compared with a nonfat control and the well-investigated supplement, calcium salts of palm FA. We hypothesized that the calcium salts of palm FA supplement would have higher digestibility than the TG supplement but that the differences would be smaller than previously reported in the literature due to differences in the FA profile of the TG supplement. Additionally, we postulated that the TG supplement would increase milk FA yield to a greater extent than the calcium salts of palm FA supplement due to differences in the FA profile of the supplemental fats.

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). Fifteen mid-lactation Holstein cows at the Michigan State University Dairy Field Laboratory were randomly assigned to treatment sequence in replicated 3×3 Latin squares with 21-d periods. All animals received a common diet with no supplemental fat during a 14-d preliminary period to obtain baseline values. At the beginning of the trial, mean ($\pm$ SD) DIM, BW, and milk yield were 139 ± 39 d, 690 ± 44 kg, and 51.4 ± 4.5 kg/d, respectively.

Treatments were a control diet (**CON**; no supplemental fat) and 1.5% of FA added either as a palmitic acid-enriched TG supplement (**PA-TG**) or as calcium salts of palm FA supplement (**Ca-FA**). The fat supplements replaced soyhulls in the diets (Table 1). Both fat-

supplemented diets were balanced for glycerol concentration; glycerol was not added to the CON treatment. Although it is important to consider possible different metabolic fates for the glycerol fed in the diet and that released from TG in the intestine, it is unlikely that it affected our results due to the very small amount required to balance glycerol intake between PA-TG and Ca-FA. The DM concentration of forages was determined twice weekly, and diets were adjusted when necessary. Cows were housed in tie-stalls throughout the entire experiment and milked twice daily (0400 and 1500 h). Access to feed was blocked from 0800 to 1000 h for collection of orts and offering of new feed. Cows were fed 115% of expected intake at 1000 h daily. Water was available ad libitum in each stall and stalls were bedded with sawdust and cleaned twice daily.

Samples and data for production and digestibility variables were collected during the last 5 d of each treatment period (d 17 to 21). Feed ingredients and orts were collected daily and composited by period and analyzed for DM, NDF, CP, starch, and FA contents as described by Boerman and Lock (2014). Fecal (500 g) samples were collected every 15 h, resulting in 8 samples per cow per period, and nutrient digestibility was calculated using indigestible NDF (iNDF) as an internal marker as previously described (Piantoni et al., 2013). Fat supplements were composited by period and chemical composition and particle size distribution determined (de Souza et al., 2017; Supplemental Table S1; <https://doi.org/10.3168/jds.2017-13560>). Individual milk samples were analyzed for fat, true protein, and lactose by mid-infrared spectroscopy (AOAC, 1990; method 972.160), and for FA using GLC (Lock et al., 2013). Yields of 3.5% FCM, ECM, and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each collection period. Body weight was measured 3 times per week, and 4 trained investigators determined BCS on a 5-point scale in 0.25-point increments on the last day of each period (Wildman et al., 1982). We determined energy partitioning using the procedures described by Boerman et al. (2015b). Data were used to calculate milk energy output and body tissue gain throughout each treatment period. Milk energy output (Mcal/d) was calculated according to NRC (2001): 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 5-d sampling period. Mean daily BW change (Δ BW, kg/d) was calculated for each cow within period by linear regression after 2 iterations of removing outliers. Energy partitioned to body tissue (Mcal/d) was estimated according to NRC (2001): body tissue gain (Mcal/d) = $[(2.88 + 1.036 \times \text{BCS}) \times$

ΔBW], where BCS was the average BCS for each cow during a 21-d period. Apparent NE_L concentration of the diet was calculated from the digestibility results, as described by Harvatine and Allen (2006).

Data were analyzed using the MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). The statistical model included the fixed effect of treatment and period, as well as 2-way interactions between these factors, and the random effects of treatment sequence and cow nested within sequence. The interaction between period and treatment was initially included in the model and removed because $P > 0.20$ for all variables. Two preplanned contrasts were used to evaluate (1) the effect of fat-supplemented treatments [CON vs. fat-supplemented treatments; $1/2$ (PA-TG + Ca-FA)], and (2) the effect of fat supplement (PA-TG vs. Ca-FA). Contrasts were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Overall, fat supplementation did not affect DMI compared with CON ($P > 0.10$; Table 2), but Ca-FA reduced DMI by 0.90 kg/d compared with PA-TG ($P < 0.05$). This is supported by several reviews that observed that feeding calcium salts of palm FA reduced DMI (Allen, 2000; Rabiee et al., 2012; Weld and Armentano, 2017). The effects of fat supplementation on DMI vary according to the source of fat fed (Rabiee et al., 2012), and the hypophagic effect of feeding fat seems to be more pronounced for unsaturated than for saturated FA supplements (Harvatine and Allen, 2006), with DMI decreasing linearly as the degree of unsaturation increases (Pantoja et al., 1994). The more pronounced decrease in DMI for unsaturated FA is likely mediated in part by increased secretion of gut peptides related to satiety (i.e., cholecystokinin and glucagon-like peptide 1; Relling and Reynolds, 2007; Bradford et al., 2008). We observed that fat supplementation reduced NDF

Table 1. Ingredient and nutrient composition of treatment diets

Item	Treatment ¹		
	CON	PA-TG	Ca-FA
Ingredient, % of DM			
Corn silage	22.1	22.1	22.1
Alfalfa silage	15.3	15.3	15.3
Wheat straw	4.60	4.60	4.60
Ground corn	12.0	12.0	12.0
High-moisture corn	12.0	12.0	12.0
Soybean meal	14.3	14.3	14.3
Soyhulls	9.39	7.70	7.94
Whole cottonseed	7.0	7.0	7.0
Palmitic acid triglyceride supplement ²	—	1.69	—
Ca-salts of palm FA supplement ³	—	—	1.80
Glycerin	—	—	0.15
Mineral and vitamin mix ⁴	2.0	2.0	2.0
Limestone	0.60	0.60	0.10
Sodium bicarbonate	0.71	0.71	0.71
Nutrient composition, % of DM ⁵			
DM	58.6	58.6	58.6
NDF	31.0	29.9	30.1
CP	16.7	16.6	16.6
Starch	26.0	26.0	25.9
FA	3.37	4.82	4.84
16:0	0.55	1.68	1.22
18:0	0.08	0.16	0.16
<i>cis</i> -9 18:1	0.59	0.79	1.17
<i>cis</i> -9, <i>cis</i> -12 18:2	1.72	1.75	1.85
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.19	0.19	0.19

¹CON = control, no addition of supplemental fatty acids; PA-TG = 1.5% of fatty acids added as a palmitic acid-enriched triglyceride supplement; Ca-FA = 1.5% of fatty acids added as a calcium salts of palm fatty acid supplement.

²BergaFat T300 (Berg + Schmidt GmbH & Co. KG, Hamburg, Germany). The supplement contained (g/100 g of fatty acid) 1.1 of C14:0, 76.8 of C16:0, 5.05 of C18:0, 13.6 of *cis*-9 C18:1, and 89.8% total fatty acids.

³Megalac (Church & Dwight Co. Inc., Princeton, NJ). The supplement contained (g/100 g of fatty acid) 0.92 of C14:0, 45.4 of C16:0, 0.69 of C18:0, 38.1 of *cis*-9 C18:1, and 85% total fatty acids.

⁴Vitamin 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, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.

⁵Expressed as percent of as fed.

Table 2. Nutrient intake, nutrient digestibility, BW, BCS, and calculated energy for cows fed treatment diets (n = 15)

Variable	Treatment ¹			SEM	Contrast ²	
	CON	PA-TG	Ca-FA		CON vs. FS	PA-TG vs. Ca-FA
Intake, kg/d						
DM	29.5	29.6	28.7	0.54	0.323	0.044
NDF	9.10	8.81	8.62	0.16	<0.01	0.162
CP	4.91	4.91	4.76	0.09	0.253	0.031
Total FA	1.01	1.44	1.41	0.08	<0.01	0.233
16-carbon	0.19	0.53	0.49	0.08	<0.01	<0.01
18-carbon	0.77	0.87	1.00	0.16	<0.01	<0.01
Digestibility, %						
DM	63.7	66.5	65.8	0.52	<0.01	0.233
NDF	36.7	41.4	39.8	0.81	<0.01	0.092
Total FA	74.7	70.1	78.8	1.25	0.526	<0.01
16-carbon	73.1	59.2	80.9	1.84	0.236	<0.01
18-carbon	76.8	79.1	78.6	1.14	0.041	0.761
BW, kg	711	712	718	14.1	0.191	0.162
BW change kg/d	0.61	0.62	0.78	0.14	0.182	0.088
BCS	3.15	3.17	3.25	0.05	0.099	0.072
BCS change	0.05	0.06	0.12	0.03	0.252	0.023
Energy						
NE _L intake, Mcal/d ³	50.8	53.0	52.2	0.69	<0.01	0.013
Milk ⁴	32.3	34.1	33.1	0.73	<0.01	0.050
Body tissue gain ⁵	4.9	4.9	5.5	0.11	<0.01	<0.01
Maintenance ⁶	13.3	13.9	13.3	0.30	0.434	0.135
Partitioning, as % energy intake						
Milk	64.3	64.4	63.9	1.41	0.796	0.183
Body tissue gain	9.61	9.30	10.5	0.32	0.021	<0.01
Maintenance	26.1	26.1	25.5	0.57	0.264	0.281

¹CON = control, no addition of supplemental fatty acids; PA-TG = 1.5% of fatty acids added as a palmitic acid-enriched triglyceride supplement; Ca-FA = 1.5% of fatty acids added as a calcium salts of palm fatty acid supplement.

²*P*-values associated with contrasts: CON vs. FS = *P*-value associated with differences between control and fat-supplemented treatments [(PA-TG + Ca-FA)/2]; PA-TG vs. Ca-FA = *P*-value associated with differences between PA-TG and Ca-FA treatments.

³From digestibility equations (NRC, 2001) according to Harvatine and Allen (2006) based on nutrient digestibility results collected during the last 5 d of each treatment period.

⁴From NRC (2001) equations: Milk energy = kg of milk × [(0.0929 × milk fat %) + (0.0563 × milk protein %) + (0.0395 × milk lactose %)].

⁵From NRC (2001) equations: Body tissue gain (Mcal/d) = [(2.88 + 1.036 × BCS) × mean daily change in BW].

⁶From NRC (2001) equations: 0.08 × BW^{0.75}.

intake by 0.39 kg/d compared with CON ($P < 0.01$), mainly a result of the lower NDF content in these diets due to the removal of soyhulls in the fat-supplemented treatments. As expected, fat-supplemented treatments increased total FA intake (0.42 kg/d) compared with CON ($P < 0.01$). The PA-TG treatment increased 16-carbon FA intake (0.04 kg/d) compared with Ca-FA ($P < 0.01$), whereas Ca-FA increased 18-carbon FA intake (0.13 kg/d) compared with PA-TG ($P < 0.01$). Fat-supplemented treatments increased NDF digestibility compared with CON ($P < 0.01$; 3.90 percentage units). In a recent meta-analysis, Weld and Armentano (2017) observed that calcium salts of palm FA did not affect NDF digestibility, whereas saturated prilled fat containing a mixture of C16:0 and C18:0 increased NDF digestibility. The increase in NDF digestibility with Ca-FA might be associated with the decrease in DMI in this treatment. Compared with Ca-FA, PA-TG tended to increase NDF digestibility ($P < 0.10$; 1.60 percentage units), which is consistent with previous

studies feeding palmitic acid-enriched FA supplements (de Souza et al., 2017; Rico et al., 2017). The increase in NDF digestibility with C16:0 supplementation may be associated with an increase in retention time driven by an increase in cholecystokinin secretion (Piantoni et al., 2013) or improvements in growth of *Butyrivibrio* bacteria in the rumen (Hackmann and Firkins, 2015).

Compared with CON, fat supplementation increased 18-carbon FA digestibility ($P < 0.05$; 2.0 percentage units), whereas it did not affect digestibility of total or 16-carbon FA (both $P > 0.10$). Under normal conditions, most FA reaching the intestine of ruminants are free fatty acids (Doreau and Chilliard, 1997), and therefore lipase activity is lower than in nonruminants (Moore and Christie, 1984). Previous studies have reported lower FA digestibility for fat supplements fed as TG than for those fed as FA supplements (Elliott et al., 1999; Weiss and Wyatt, 2004). With that, some authors postulated that lipolysis in the small intestine might be a rate-limiting step to FA digestion (Pantoja

et al., 1995). Because several lipases are active only at the distal end of the small intestine, they may be unable to hydrolyze large amounts of duodenal TG when these supplements are fed, therefore possibly limiting intestinal digestibility (Jenkins and Jenny, 1992). In our study, we observed that PA-TG reduced total FA digestibility by 8.70 percentage units compared with Ca-FA ($P < 0.01$). This reduction in total FA digestibility by PA-TG was a result of the decrease in 16-carbon FA digestibility ($P < 0.01$; 21.7 percentage units). However, the decrease in FA digestibility was smaller than that previously reported in other studies that fed saturated TG supplements, in which total FA digestibility ranged from 33 to 62% (e.g., Pantoja et al., 1995; Elliott et al., 1999; Weiss and Wyatt, 2004). This difference may be associated with differences in the FA profile of the supplements being evaluated and potentially differences in lipolysis in the rumen. The supplemental TG in the previously described studies contained high concentrations of SFA, mainly C18:0 (31 to 58%), whereas the TG supplement in our study contained only ~5% C18:0. In a meta-analysis evaluating the digestibility of individual FA using duodenally cannulated cows, Boerman et al. (2015a) determined that as the amount of C18:0 reaching the duodenum increased, the digestibility of C18:0 decreased linearly across fat-supplemented and non-fat-supplemented diets encompassing a wide range of C18:0 duodenal flows. In addition, Johnson et al. (1974) reported lower intestinal hydrolysis in sheep for tallow compared with coconut oil, olive oil, or corn oil. This was attributed to lower lipase activity resulting from lower solubility and poor emulsification of tallow due to its FA profile. Therefore, the lower solubility of TG enriched with C18:0 may increase the need for emulsification compounds to form micelles and possibly reduce the time of action of intestinal lipases and for FA absorption. Alternatively, although we did not measure it in our study, our PA-TG supplement could have been partially hydrolyzed in the rumen, resulting in less intact TG reaching the intestine compared with previous studies that fed partially or fully hydrogenated tallow. This hypothesis deserves further investigation. Similar to our results, Weiss et al. (2011) observed that total FA digestibility was reduced by ~8 percentage points when a saturated TG supplement containing 72% C16:0 and 5% C18:0 was fed compared with a control diet. By using a Lucas test, we estimated true digestibility of the fat supplements in our study as 62 and 81% for PA-TG and Ca-FA, respectively (Supplemental Figure S1; <https://doi.org/10.3168/jds.2017-13560>). Our result for the PA-TG supplement is similar to the value estimated (56%) by Weiss et al. (2011) for their saturated TG supplement with a similar FA profile.

Interestingly, we also observed that Ca-FA had the highest FA digestibility, including 16- and 18-carbon FA. Recently, Rico et al. (2017) observed that 16-carbon FA digestibility was reduced when feeding a palmitic acid-enriched supplement (85% C16:0) to a greater extent in a soyhulls basal diet than in a whole cottonseed basal diet. Although total flow of FA at the duodenum affects FA digestibility (Boerman et al., 2015a), these findings support the hypothesis that the profile of FA entering the duodenum is a critical factor affecting FA digestibility. Additionally, previous results have suggested that *cis*-9 C18:1 has greater digestibility than C18:0 and C16:0 (Boerman et al., 2015a), and *cis*-9 C18:1 is thought to have amphiphilic properties (Moate et al., 2004). This is supported by Freeman (1969), who examined the amphiphilic properties of polar lipid solutes and found that *cis*-9 C18:1 had a positive effect on the micellar solubility of C18:0. It is important to point out that even though *cis*-9 C18:1 was the main FA in our Ca-FA supplement, it is likely that this treatment increased rumen outflow of other 18-carbon FA, including C18:0. Additional research is required to understand the mechanisms by which *cis*-9 C18:1 may increase digestibility of other FA.

Compared with CON, fat-supplemented treatments increased milk yield by 1.05 kg/d ($P < 0.05$; Table 3), 3.5% FCM by 2.20 kg/d ($P < 0.01$), and ECM by 1.80 kg/d ($P < 0.01$). Also, PA-TG increased milk fat yield ($P < 0.05$; 50 g/d) and milk energy output ($P < 0.05$; 1.0 Mcal/d), and tended to increase milk fat content ($P < 0.10$; 0.07 percentage units) and ECM ($P < 0.10$; 1.0 kg/d) compared with Ca-FA. These results are in agreement with most of our short-term studies feeding palmitic acid-enriched free FA supplements (fed at 1.5 to 2.0% of diet DM) that have indicated increases in milk fat yield of ~100 g/d compared with nonfat control diets (Piantoni et al., 2013; Lock et al., 2013; de Souza et al., 2016). The fat-supplemented treatments reduced the concentration (2.90 g/100 g of FA) and yield (23 g/d) of de novo ($P < 0.05$) milk FA and increased the concentrations (2.15 and 0.75 g/100 g of FA, respectively) and yields (43 and 52 g/d, respectively) of mixed and preformed ($P < 0.05$) milk FA compared with CON. The PA-TG treatment increased the yield of 16-carbon milk FA by 66 g/d compared with Ca-FA ($P < 0.01$). The increase in 16-carbon milk FA with PA-TG was due to the increase in concentration and yield of C16:0 ($P < 0.01$; Supplemental Tables S2 and S3; <https://doi.org/10.3168/jds.2017-13560>). Also, Ca-FA reduced the concentration ($P < 0.01$; 1.20 g/100 g of FA) and tended to reduce the yield of de novo ($P < 0.10$; 20 g/d) milk FA compared with PA-TG. In contrast, compared with PA-TG, Ca-FA increased the concentration (3.10 g/100 g of FA) and yield (60 g/d) of preformed milk

Table 3. Milk yield, milk composition, and milk fatty acids (FA) for cows fed treatment diets (n = 15)

Variable	Treatment ¹			SEM	Contrast ²	
	CON	PA-TG	Ca-FA		CON vs. FS	PA-TG vs. Ca-FA
Milk yield, kg/d						
Milk	48.3	49.7	48.9	1.06	0.049	0.237
3.5% FCM ³	47.1	49.7	48.9	1.27	<0.01	0.102
ECM ⁴	47.1	49.4	48.4	1.19	<0.01	0.083
Milk composition						
Fat, kg/d	1.64	1.74	1.69	0.03	<0.01	0.048
Fat, %	3.40	3.57	3.50	0.04	<0.01	0.098
Protein, kg/d	1.47	1.51	1.46	0.04	0.503	0.059
Protein, %	3.04	3.05	2.99	0.05	0.146	<0.01
Lactose, kg/d	2.31	2.40	2.37	0.06	0.029	0.337
Lactose, %	4.78	4.85	4.86	0.04	0.086	0.986
Milk FA concentration, ⁵ g/100 g of FA						
De novo	26.4	24.1	22.9	0.55	<0.01	<0.01
16-carbon	32.7	35.9	33.8	0.47	<0.01	<0.01
Preformed	40.9	40.1	43.2	0.53	0.047	<0.01
Milk FA yield, ⁵ g/d						
De novo	404	392	372	17.8	0.026	0.085
16-carbon	500	576	510	26.5	<0.01	<0.01
Preformed	618	640	700	28.4	<0.01	<0.01
FCM:DMI	1.60	1.68	1.71	0.04	<0.01	0.282

¹CON = control, no addition of supplemental fatty acids; PA-TG = 1.5% of fatty acids added as a palmitic acid-enriched triglyceride supplement; Ca-FA = 1.5% of fatty acids added as a calcium salts of palm fatty acid supplement.

²*P*-values associated with contrasts: CON vs. FS = *P*-value associated with differences between control and fat-supplemented treatments [(PA-TG + Ca-FA)/2]; PA-TG vs. Ca-FA = *P*-value associated with differences between PA-TG and Ca-FA 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)].

⁵De novo FA originate from mammary de novo synthesis (<16 C), preformed FA originated from extraction from plasma (>16 C), and 16-C FA originate from both sources (C16:0 plus *cis*-9 C16:1).

FA (both $P < 0.01$) due to the increase in the yield of several 18-carbon FA in milk fat, including *cis*-9 C18:1 ($P < 0.01$), *cis*-9,*cis*-12 C18:2 ($P < 0.01$), *cis*-9,*trans*-11 C18:2 ($P < 0.05$), and *cis*-9,*cis*-12,*cis*-15 C18:3 ($P < 0.05$; Supplemental Table S3; <https://doi.org/10.3168/jds.2017-13560>). The substitution effect of preformed for de novo milk FA has been reported previously (He and Armentano, 2011; He et al., 2012), in which the reduction in yield of de novo milk FA was compensated for by an increase in the yield of preformed milk FA when fat supplements were fed.

Compared with CON, fat-supplemented treatments did not affect milk protein content and yield ($P > 0.10$) but tended to increase content (0.08 percentage units) and yield (70 g/d) of milk lactose ($P < 0.10$). The Ca-FA treatment decreased milk protein content by 0.06 percentage units ($P < 0.01$) and tended to decrease milk protein yield by 45 g/d ($P < 0.10$) compared with PA-TG. Supplemental fat often reduces milk protein content compared with nonfat controls (Rabiee et al., 2012) possibly due to decreased glucose availability, development of insulin resistance, increased efficiency of milk production, reduced plasma somatotropin, and reduced amino acid supply to the mammary gland (Cant et al., 1993; Wu and Huber, 1994). In our study,

the reduction in milk protein concentration with the Ca-FA treatment compared with PA-TG was likely due to the lower DMI and CP intake. The reduction in DMI would suggest less MP available for milk protein synthesis (Weiss et al., 2011).

Compared with CON, fat-supplemented treatments increased calculated NE_L intake by 1.80 Mcal/d, milk energy output by 1.30 Mcal/d, and energy in body reserves by 0.30 Mcal/d (all $P < 0.01$; Table 2). The Ca-FA treatment increased energy allocated to body reserves (0.60 Mcal/d) and energy partitioning toward body reserves (1.20 percentage units; both $P < 0.01$) and tended to increase BW change (0.16 kg/d; $P < 0.10$) and increased BCS change (0.06 units; $P < 0.05$) compared with PA-TG. Also compared with PA-TG, Ca-FA increased the concentration and yield of *trans*-10 C18:1 in milk fat. Similarly, Boerman et al. (2015b) observed a positive correlation between milk fat *trans*-10 C18:1 content and change in BCS; however, the increase in milk *trans*-10 C18:1 was not associated with changes in milk fat yield. The Ca-FA treatment provided a higher load of UFA in the diet, which likely overcome normal rumen biohydrogenation capacity and altered biohydrogenation pathways. This may be associated with repartitioning of energy by reducing

milk energy output and increasing body fat reserves. Although in our study we did not detect levels of *trans*-10,*cis*-12 C18:2 in milk fat for most of our samples, Harvatine et al. (2009) reported that during abomasal infusions of *trans*-10,*cis*-12 C18:2, the expression of lipogenic enzymes was downregulated in mammary tissue and upregulated in adipose tissue. Conversely, Urrutia and Harvatine (2017) observed reduced lipogenic capacity of adipose tissue explants without changes in gene expression of key lipogenic enzymes during 4 d of *trans*-10,*cis*-12 C18:2 infusion of low-producing cows, which suggested that energy partitioning under milk fat depression-induced conditions may be affected by physiological state. Further studies are needed to evaluate the association between changes in biohydrogenation pathways and partitioning of energy across different tissues under different physiological conditions, as well as changes in energy partitioning driven by fat supplementation in long-term studies. Additionally, it is important to note that our design used 21-d periods, and changes in BW in short-term studies may be confounded by differences in gut fill between diets (Weiss et al., 2011). Although this consideration is important, we measured BW 3 times per week, which likely improved the accuracy of our measurements compared with studies that only measured BW at the beginning and end of the study.

In conclusion, our results demonstrate that calcium salts of palm FA supplements have higher FA digestibility than palmitic acid-enriched TG supplements. Although the palmitic acid-enriched TG supplement reduced FA digestibility, the reduction was smaller than previously reported in the literature for highly saturated TG supplements with higher concentrations of C18:0, less total UFA, and lower iodine value. Feeding a palmitic acid-enriched triglyceride supplement increased milk energy output due to increased yields of milk and milk fat, whereas feeding a calcium salts of palm FA supplement increased FA digestibility and energy partitioned to body reserves.

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