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Effects of supplementing Holstein cows with soybean oil compared with palmitic acid–enriched triglycerides on milk production and nutrient partitioning

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ABSTRACT

Both insulin and *trans*-10,*cis*-12 C18:2 (t10c12CLA) can be increased by high-starch diets; thus, it is difficult to determine whether insulin or t10c12CLA mediates nutrient partitioning toward body tissues during milk fat depression. To minimize insulin secretion while manipulating t10c12CLA levels, diets supplemented with palmitic acid–enriched triglycerides and soybean oil were fed to cows. Thirty-two Holstein cows (93 ± 35 d in milk) were included in the crossover experiment with each treatment period being 28 d. Treatment diets contained 25% neutral detergent fiber, 32% starch, 18% crude protein, and 4.6% fatty acids (dry matter basis). Treatment diets contained either palmitic acid–enriched triglycerides (2.5% dry matter, BergaFat T-300, Berg + Schmidt America LLC, Libertyville, IL; PAT) or soybean oil (2.5% dry matter; SBO). Cows were blocked by milk yield, body weight, and parity, and then randomly assigned to 1 of 2 treatment sequences (PAT-SBO or SBO-PAT). Cows fed PAT produced milk with only 3.1% fat, indicating milk fat depression; SBO decreased fat content further to only 2.4%. No effect of treatment was observed on dry matter intake, apparent net energy intake, milk yield, body condition score, or fat thickness over the rump and rib. However, compared with PAT, SBO decreased fat-corrected milk yield, energy-corrected milk yield, milk fat yield, de novo fatty acids, and 16-carbon fatty acid yield, whereas SBO increased body weight gain. Neutral detergent fiber digestibility tended to be lower in SBO, whereas fatty acid digestibility was higher. Additionally, the concentration of plasma insulin, nonesterified fatty acids, and triglycerides, and milk metabolites (*trans*-10 C18:1 and t10c12CLA) were all higher in SBO. In conclusion, with similar dietary starch content, the diet containing palmitic acid–enriched triglycerides partitioned more energy toward milk synthesis, whereas the diet containing soybean oil partitioned more energy toward body tissue gain.

Key words: milk fat depression, insulin, *trans*-10,*cis*-12 conjugated linoleic acid

INTRODUCTION

In cows suffering milk fat depression (**MFD**), more nutrients are stored in adipose tissues, whereas fewer are used for milk fat synthesis (Van Soest, 1963; Bauman and Griinari, 2001; Boerman et al., 2015). Insulin and CLA have been well recognized as key mediators in nutrient partitioning during lactation. The role of plasma insulin in nutrient partitioning is well established (Bauman and Griinari, 2001). Ruminal CLA isomers have also been documented as key regulators in nutrient partitioning during MFD. Plasma insulin prevents lipolysis and stimulates lipid synthesis in adipose tissues (Vernon, 2005; Bauman et al., 2011), and decreases fatty acid (**FA**) availability for milk fat synthesis (McClymont and Vallance, 1962); therefore, insulin favors energy partitioning toward body tissue gain instead of milk synthesis. Specific CLA isomers, especially *trans*-10,*cis*-12 C18:2 (**t10c12CLA**), are known as potent inhibitors of milk fat synthesis (Bauman et al., 2011). Harvatine et al. (2009) reported that milk energy output decreased the expression of lipogenic genes in adipose tissue increased in cows suffering CLA-induced MFD. Thus, t10c12CLA also favors energy flow toward body tissue gain instead of milk synthesis. Both t10c12CLA and insulin are potent regulators of energy partitioning in lactating dairy cows; however, to our knowledge, no studies have tried to separate the effect of these.

In Boerman et al. (2015), cows fed a high-starch diet exhibited MFD and significant body tissue gain, along with higher concentrations of plasma insulin and milk *trans*-10 C18:1 (a robust marker of alterations in ruminal biohydrogenation and inhabitation of milk fat synthesis; Bauman et al., 2011). As high-starch diets

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were fed to cows to induce MFD in Boerman et al. (2015), both insulin and t10c12CLA were increased in that study, and thus we were not able to separate the effects of insulin and t10c12CLA. Both mechanisms may have caused the diet-induced MFD and increased BW gain. Whether it was insulin, t10c12CLA, or both that altered nutrient partitioning was not clear. One way to better understand the effect of t10c12CLA on nutrient partitioning is to increase t10c12CLA production while minimizing insulin secretion. To do this, we fed cows 2 isoenergetic and isolipid diets with similar starch content but supplemented with either palmitic acid–enriched triglycerides or soybean oil. With these 2 supplements, we could cause MFD with the diet containing soybean oil while minimizing rumen issues and promoting milk fat synthesis with the diet containing palmitic acid–enriched triglycerides. We hypothesized that feeding mid-lactation cows a diet containing soybean oil would enhance ruminal CLA production, cause almost no change in insulin concentration, and partition more energy toward body tissue.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University. Thirty-two mid-lactation Holstein cows (14 primiparous and 18 multiparous) were included in a crossover design experiment with two 28-d treatment periods. Cows were blocked by milk yield, parity, and BW, and randomly assigned to one of the treatment sequences. Mean DIM, BW, and milk yield were 93 ± 35 d, 668 ± 61 kg, and 46 ± 11 kg/d (mean ± SD) at the start of the experiment, respectively. Cows were housed in individual tiestalls and milked twice daily. Water was available ad libitum. Feed was offered once daily at 1200 h at 115% of expected intake.

Treatment diets were supplemented with either palmitic acid–enriched triglycerides [2.5% DM, BergaFat T-300 (Berg + Schmidt America LLC, Libertyville, IL); **PAT**] or soybean oil (2.5% DM; **SBO**; Table 1). Forages were corn silage and alfalfa silage. Mineral and vitamins were formulated according to NRC (2001). The feed ingredients and nutrient composition of the TMR diets are described in Table 1. Diets were adjusted for changes in forage DM concentration twice weekly when necessary.

Sample Collection and Analysis

Milk yield was recorded daily and milk samples at each milking were collected for 2 consecutive days each week throughout the study for the calculation of energy partitioning. Data and samples for production performance, digestibility, and plasma metabolites/hormones were intensively collected during the final 5 d of each treatment period. Samples of 10 consecutive milkings were collected from individual cows. Two aliquots of the milk samples from each cow were collected at each milking. One of the aliquots was analyzed for fat, protein, and lactose using infrared spectroscopy by Michigan DHIA (Grand Ledge, MI). The other aliquot was stored at −20°C; the 10 samples were subsequently composited based on milk fat yield (d 24–28 of each

Table 1. Ingredients and nutrient composition of treatment diets[1,2,3]

Item	Treatment	
	PAT	SBO
Ingredient, % of DM		
Corn silage	29.0	29.1
Alfalfa silage	14.1	14.1
Cottonseed, whole	5.3	5.3
Corn, ground	10.5	10.5
Corn, high moisture	18.5	18.5
Soybean meal	16.7	16.7
C16:0-enriched fat supplement[4]	2.5	—
Soybean oil	—	2.5
Vitamin and mineral premix[5]	2.0	2.0
Limestone	0.7	0.7
Sodium bicarbonate	0.7	0.7
Forage:concentrate	43:57	43:57
Nutrient composition, % of DM		
DM[6]	57.1	57.1
NDF	25.0	25.1
Forage NDF	18.1	18.2
CP	18.2	17.9
Starch	31.6	31.9
Fatty acids	4.79	4.40
16-Carbon fatty acids	2.19	0.64
18-Carbon fatty acids	2.48	3.65
Apparent NE_L,[7] Mcal/kg	1.64	1.66

[1]Experimental diets fed to 32 cows in a crossover design within 28-d periods.

[2]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or soybean oil (SBO) on a DM basis.

[3]Nutrient composition was determined from feed ingredients sampled during the last 5 d of each 28-d experimental period.

[4]BergaFat T-300 (Berg + Schmidt America LLC, Libertyville, IL).

[5]The vitamin and mineral premix was designed to meet the mineral and vitamin requirements of lactating cows as set forth by NRC (2001). The premix mix contained 34.1% dry ground shelled corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos (Mosaic, Tampa, FL), 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.

[6]DM was expressed as a percentage of as fed.

[7]Mean apparent net energy concentration of diets, based on average cow performance. For each diet, diet NE_L = the average of (MilkE + 0.08 × MBW + ΔBodyE)/DMI for all cows on the diet, where MilkE is net energy used for milk synthesis, MBW is metabolic body weight ($BW^{0.75}$), and ΔBodyE is net energy captured in body tissue.

period). Milk lipids were extracted, and FAME were prepared using sodium methoxide solution in methanol and quantified using GLC according to our methods described previously (Lock et al., 2013). Individual FAME were identified by comparison of retention times with known FAME standards. Yield 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).

Cows were weighed 3 times per week immediately following afternoon milkings throughout the study. Body condition score for each cow, calculated as the average score of 3 trained investigators, was recorded on a 5-point scale at the end of each period. On the last day of each period, subcutaneous fat thickness was measured at the 12th intercostal space for rib fat and the sacral region between the tuber coxae (hooks) and tuber ischia (pins) for rump fat via ultrasound scanning. The National Centralized Ultrasound Processing Lab (Ames, IA) analyzed the ultrasound images, and the change in subcutaneous fat thickness was calculated as the difference between consecutive measurements.

During the last 5 d of each experimental period, samples of feed ingredients (~0.5 kg) and orts (12.5%) for individual cows were collected daily and composited for each cow by period. Samples of feces were collected every 15 h to provide 8 samples per cow during the last 5 d of each period (1200, 0300, 1800, 0900, 2400, 1500, 0600, and 2100 h). Fecal samples were stored at −20°C after collection until being dried. Samples of feed ingredients, orts, and feces were dried using a forced-air oven (55°C for 72 h) before being ground through a Wiley mill (2-mm screen for cottonseed, 1-mm screen for other ingredients, orts, and fecal sample; Arthur H. Thomas Co., Philadelphia, PA). Following grinding, individual fecal samples were composited for each cow by period, on an equal DM basis.

Feed ingredients, orts, and feces were analyzed for NDF, indigestible NDF, and FA. The NDF and indigestible NDF were measured using methods described by Mertens (2002) and Goering and Van Soest (1970), respectively. Indigestible NDF was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986). The concentration of FA in feed ingredients, orts, and feces were determined as described by Lock et al. (2013). Additionally, feed ingredients were analyzed for CP and starch (AOAC, 1990) by Cumberland Valley Analytical Services Inc. (Hagerstown, MD).

Blood was sampled at the same time as fecal collection, via coccygeal venipuncture into three 6-mL evacuated tubes. Two tubes contained potassium EDTA and the third one contained potassium oxalate with sodium fluoride as glycolytic inhibitors. Immediately after collection, plasma was separated from red blood cells by centrifugation at 2,000 × *g* for 15 min at 4°C and then stored at −20°C until composited into one sample per cow per period. Commercial kits were used to determine plasma concentration of nonesterified fatty acids [NEFA-HR (2) kit, Wako Chemicals, Richmond, VA], insulin (Bovine Insulin ELISA, Mercodia, Uppsala, Sweden), and triglycerides (L-Type triglyceride M kit, Wako Chemicals). Plasma glucose concentration was determined by a glucose oxidase method (PGO Enzyme Product No. P7119, Sigma Chemical Co.).

Calculations

Weekly milk yields of fat, protein, and lactose obtained from 4 consecutive milkings, weekly BW, and period BCS were included in the energy partitioning calculations. Milk energy output, metabolic BW, and body tissue gain throughout treatment periods were calculated based on the data above.

Milk energy output (MilkE; Mcal/d) for each cow was estimated by the following equation (NRC, 2001; from Equation 2–15):

$$\text{MilkE} = 9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)},$$

where each component was the average output of each cow during the 28-d period. Metabolic BW (MBW; $kg^{0.75}$) was estimated as $BW^{0.75}$, where BW was the mean BW of each cow during the 28-d period.

Energy for maintenance (MaintE; Mcal/d) for each cow was estimated as

$$\text{MaintE} = 0.08 \times \text{MBW}.$$

Mean daily BW change (ΔBW; kg/d) was calculated for each cow within the treatment period by linear regression after 2 rounds of removing outliers in the data; an outlier was any BW >3.5 SD from the regression line.

Energy expended for body tissue gain (ΔBodyE; Mcal/d) was estimated by an equation derived from NRC (2001; Table 2, 3, 4, and 5):

$$\Delta\text{BodyE} = (2.88 + 1.036 \times \text{BCS}) \times \Delta\text{BW},$$

where BCS was the average BCS for each cow during the 28-d period.

Energy partitioning was predicted based on observed performance:

Table 2. Dry matter intake, milk production, milk components, and feed efficiency for cows fed treatment diets (n = 32)

Item	Treatment[1]		SEM	*P*-value[2,3]
	PAT	SBO		TRT
DMI	25.0	24.9	0.63	0.65
Milk yield, kg/d				
Milk	46.1	46.5	1.75	0.58
ECM[4]	42.6	39.8	1.56	<0.01
3.5% FCM[5]	41.9	38.1	1.60	<0.01
Milk component				
Fat, kg/d	1.35	1.11	0.05	<0.01
Fat, %	3.07	2.42	0.13	<0.01
Protein, kg/d	1.40	1.44	0.05	0.04
Protein, %	3.05	3.12	0.03	<0.01
Lactose, kg/d	2.21	2.24	0.08	0.42
Lactose, %	4.81	4.83	0.03	0.2
ECM/DMI[6]	1.67	1.53	0.04	<0.01

[1]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or 2.5% soybean oil (SBO) on a DM basis.

[2]*P*-value associated with treatment differences (PAT vs. SBO; TRT).

[3]All *P*-values for period × treatment were greater than 0.60.

[4]ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)] (Tyrrell and Reid, 1965).

[5]3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

[6]Milk:feed ratio = ECM/DMI.

$$\%\ \text{milk, maintenance, or body tissue} = \text{MilkE, MaintE, or BodyE}/(\text{MilkE} + \text{MaintE} + \Delta\text{BodyE}) \times 100,$$

where % to milk, maintenance, or body tissue was the percentage of apparent net energy partitioned to milk production, maintenance requirement, or body tissue gain, respectively.

Milk to feed ratio for each cow per period was calculated as the average daily ECM yield over the average daily DMI, where ECM = 0.327 × milk (kg) + 12.95 × fat (kg) + 7.20 × protein (kg) (Tyrrell and Reid, 1965).

Apparent diet energy content (DietNE_L; Mcal/kg) for each cow on each diet was calculated as the average NE_L divided by the average daily DMI:

$$\text{DietNE}_\text{L} = (\text{MilkE} + \text{MaintE} + \Delta\text{BodyE})/\text{DMI}.$$

Statistical Analysis

Treatment responses for production performance, production efficiency, FA profile, blood metabolites/hormones, and digestibility were analyzed using the Mixed procedure in SAS (version 9.4, SAS Institute

Table 3. Body weight, BCS, and change in subcutaneous fat thickness measurements and calculated energy values for cows fed treatment diets (n = 32)

Variable	Treatment[1]		SEM	*P*-value[2,3]
	PAT	SBO		TRT
BW	677	681	10.9	0.09
BCS	3.29	3.33	0.07	0.13
Change in BW,[4] kg/d	0.19	0.46	2.37	0.04
Change in BCS, point/28 d	0.11	0.12	0.03	0.81
Change in rump fat, mm/28 d	0.20	0.06	0.19	0.67
Change in rib fat, mm/28 d	0.33	0.57	0.18	0.45
Calculated energy value[5]				
Apparent NE_L of diet, Mcal/kg	1.64	1.66	0.03	0.51
Milk, Mcal/d	29.1	27.1	1.12	<0.01
Body tissue gain, Mcal/d	1.40	3.33	0.55	<0.01
Maintenance, Mcal/d	10.6	10.7	0.13	0.17
Partitioning[6]				
Milk, %	70.8	65.8	1.4	<0.01
Body tissue gain, %	3.4	8.1	1.5	<0.01
Maintenance, %	25.7	26.0	0.6	0.79

[1]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or 2.5% soybean oil (SBO) on a DM basis.

[2]*P*-value associated with treatment differences (PAT vs. SBO; TRT).

[3]All *P*-value for period × treatment are over 0.50.

[4]Determined by linear regression using BW measurements throughout the period.

[5]Milk (MilkE) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)]. Body tissue gain (ΔBodyE) = [(2.88 + 1.036 × BCS) × ΔBW], maintenance = 0.08 × MBW.

[6]% to milk, maintenance, or body tissue = [MilkE, 0.08 × MBW, or ΔBodyE/(MilkE + 0.08 × MBW + ΔBodyE) × 100], where MilkE is net energy used for milk synthesis, MBW is metabolic body weight, and ΔBodyE is net energy captured in body tissue.

Table 4. Nutrient intake and nutrient digestibility for cows fed treatment diets (n = 32)

	Treatment[1]			*P*-value[2,3]
Nutrient	PAT	SBO	SEM	TRT
Intake, g/d				
Total FA	1,218	1,110	29.8	<0.01
16 Carbon	548	159	8.53	<0.01
18 Carbon	620	909	13.0	<0.01
Digestibility, %				
DM	64.9	64.2	0.55	0.34
NDF	29.1	26.4	1.47	0.09
Total FA	62.2	68.1	1.26	<0.01
16 Carbon	52.4	68.5	1.51	<0.01
18 Carbon	72.9	69.9	1.34	0.04
Absorbed, g/d				
Total FA	759	756	16.2	0.32
16 Carbon	287	109	6.30	<0.01
18 Carbon	452	635	9.32	<0.01

[1]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or 2.5% soybean oil (SBO) on a DM basis.

[2]*P*-value associated with treatment differences (PAT vs. SBO; TRT).

[3]All *P*-value for period × treatment are over 0.15, except for total fatty acids (FA; $P = 0.04$) and 18-carbon FA ($P = 0.04$). In period 1, total FA digestibility was higher in SBO (68. 1% vs. 57. 4%, $P < 0.01$), whereas in period 2, total FA digestibility was similar between SBO and PAT (68.7% vs. 66.9%, $P = 0.48$). In period 1, C18 FA digestibility was similar between SBO and PAT (67.3% vs. 69.1%, $P = 0.51$), whereas in period 2, C18 FA digestibility was lower in SBO (78.6% vs. 70.7%, $P < 0.01$).

Inc., Cary, NC) according to the model $Y_{ijk} = \mu + T_i + P_j + T_i \times P_j + C_k + e_{ijk}$, where Y_{ijk} was the dependent variable, μ was the overall mean, T_i was the fixed effect of treatment (i = PAT or SBO), P_j was the fixed effect of period (j = 1 or 2), $T_i \times P_j$ was the interaction between treatment and period, C_k was the random effect of cow (k = 1, …, 32), and e_{ijk} was the residual error.

Main effects were considered significant at $P < 0.05$ and trends at $P < 0.10$. Interactions were considered significant at $P \leq 0.10$ and trends at $P \leq 0.15$. All results were expressed as least squares means and standard error of means in the tables unless otherwise specified.

Table 5. Plasma concentrations of glucose, insulin, nonesterified fatty acids (NEFA), and triglycerides (TAG) for cows fed treatment diets (n = 32)

	Treatment[1]			*P*-value[2,3]
Variable	PAT	SBO	SEM	TRT
Glucose, mg/dL	59.6	60.1	0.42	0.25
Insulin, µg/L	1.18	1.34	0.05	0.03
NEFA, µEq/L	122	137	5.3	<0.01
TAG, mg/dL	7.9	8.5	0.29	0.05

[1]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or soybean oil (SBO) on a DM basis.

[2]*P*-value associated with treatment differences (PAT vs. SBO; TRT).

[3]All *P*-values for treatment × period are over 0.70 except for glucose ($P = 0.06$). In period 1, plasma glucose concentration was lower in SBO (59.0 vs. 61.4, $P = 0.04$); in period 2, plasma glucose concentration was similar between SBO and PAT (60.2 vs. 58.9, $P = 0.04$).

RESULTS

Production Performance

Treatment did not alter milk yield ($P = 0.58$) or DMI ($P = 0.65$; Table 2). Compared with PAT, SBO decreased FCM by 3.8 kg/d ($P < 0.01$), ECM by 2.8 kg/d ($P < 0.01$), milk fat concentration by 0.65 units ($P < 0.01$), milk fat yield by 240 g/d ($P < 0.01$), and ECM per DMI by 0.14 units ($P < 0.01$). However, SBO increased milk protein concentration by 0.07 units ($P < 0.01$) and milk protein yield by 40 g/d ($P = 0.04$) compared with PAT.

Body Composition

Treatment did not alter BCS ($P = 0.13$), BCS gain ($P = 0.81$), or fat thickness over the rump ($P = 0.67$) and rib ($P = 0.45$). However, SBO increased daily BW gain by 0.27 kg/d ($P = 0.04$) compared with PAT.

Calculated Energy Values and Partitioning

Compared with PAT, SBO decreased milk energy output by 2 Mcal/d (Table 3) and increased energy deposited in body tissue by 1.93 Mcal/d ($P < 0.01$). As a percentage, SBO decreased the MilkE fraction of NE_L by 5 units ($P < 0.01$) and increased the BodyE fraction of NE_L by 4.7 units ($P < 0.01$). Based on cow performance, apparent dietary NE_L values were similar for the 2 diets ($P = 0.51$).

Digestibility

Compared with PAT, SBO did not alter DM digestibility ($P = 0.34$; Table 4), tended to reduce NDF digestibility ($P = 0.09$), and increased 16-carbon FA digestibility ($P < 0.01$). We observed treatment by period interactions for total FA digestibility and 18-carbon FA digestibility. Specifically, SBO increased total FA digestibility in period 1, with no effect in period 2; SBO decreased 18-carbon FA digestibility in period 2, with no effect in period 1.

Plasma Metabolites and Hormones

Compared with PAT, SBO increased plasma concentration of insulin by 0.16 µg/L ($P = 0.03$), nonesterified fatty acids by 15 µEq/L ($P < 0.01$), and triglycerides by 0.6 mg/dL ($P = 0.05$). We observed a treatment

by period interaction for plasma glucose, where SBO increased plasma concentration of glucose in period 1, but not in period 2 (Table 5).

Milk Fatty Acids

Milk FA yields and concentration are shown in Table 6 (<16 carbon FA are from de novo synthesis in the mammary glands; >16 carbon FA originate from extraction from plasma; 16 carbon FA are from mixed sources). Compared with PAT, SBO reduced de novo FA yield by 65 g/d ($P < 0.01$) and concentration by 1.4 percentage units ($P < 0.01$). The SBO also reduced mixed source milk FA yield by 178 g/d ($P < 0.01$) and concentration by 8.7 percentage units ($P < 0.01$). In contrast, SBO did not alter preformed milk FA yield ($P = 0.27$), but increased preformed FA concentration by 10 percentage units ($P < 0.01$). Compared with PAT, SBO increased yields of *trans*-10 C18:1 by 14.9 g/d ($P < 0.01$) and t10c12CLA by 0.11 g/d ($P < 0.01$). The SBO also increased concentration of milk *trans*-10 C18:1 by 2.05 percentage units ($P < 0.01$) and t10c-12CLA by 0.02 percentage units ($P < 0.01$). Further details regarding the yield and concentration of specific FA are shown in Supplemental Tables S1 and S2 (https://doi.org/10.3168/jds.2019-18100).

DISCUSSION

In our previous study (Boerman et al., 2015), concentrations of both plasma insulin and milk t10c12CLA were increased in cows fed high-starch diets; thus, it was difficult to differentiate the effects of t10c12CLA and insulin on production performance and energy partitioning. We acknowledge that other mechanisms might explain the nutrient partitioning in Boerman et al. (2015); however, given that effects of insulin and t10c12CLA on nutrient partitioning are well documented in prior work (Bauman and Griinari, 2001; Harvatine et al., 2009; Bauman et al., 2011), we focused on insulin and t10c12CLA. To better understand the effect of t10c12CLA, independent of insulin, we fed cows diets containing similar starch content and supplemented with either palmitic acid–enriched triglycerides or soybean oil. With these 2 supplements, we could minimize rumen issues and promote milk fat synthesis with the diet containing palmitic acid–enriched triglycerides and cause MFD with the diet containing soybean oil. These diets caused significant differences in t10c12CLA production while causing relatively small changes in insulin secretion, which might help us distinguish the effect of t10c12CLA from that of insulin.

As seen in other MFD studies (Tyrrell and Moe, 1972; Boerman et al., 2015), SBO partitioned more energy toward body tissue gain instead of milk fat synthesis compared with PAT. In our study, milk energy output decreased 2 Mcal/d, which was all from decreased milk fat output, and estimated body tissue energy gained was 2 Mcal/d. This calculated body tissue energy gain was based on BW gain and was not supported by a gain in BCS or back fat thickness. We calculated BW change for each cow within treatment periods by linear

Table 6. Milk fatty acid (FA) yields and concentration of cows fed treatment diets (n = 32)[1]

	Treatment[2]			*P*-value[3,4]
Item	PAT	SBO	SEM	TRT
FA yield, g/d				
De novo[5]	292	227	11.2	<0.01
Mixed	493	315	14.1	<0.01
Preformed	479	499	17.5	0.27
trans-10 C18:1	26.6	41.5	4.62	<0.01
trans-10,*cis*-12 C18:2	0.159	0.271	0.03	<0.01
FA concentration, g/100 g				
De novo	22.3	20.9	0.43	<0.01
Mixed	39.2	30.5	0.67	<0.01
Preformed	38.5	48.5	0.88	<0.01
trans-10 C18:1	2.50	4.55	0.5	<0.01
trans-10,*cis*-12 C18:2	0.014	0.028	0.003	<0.01

[1]Samples for milk FA were collected during the last 5 d of each treatment period (d 24 to 28).

[2]Treatments contained 2.5% added palmitic acid–enriched triglyceride (PAT) or soybean oil (SBO) on a DM basis.

[3]*P*-value associated with treatment differences (PAT vs. SBO; TRT).

[4]All *P*-values for treatment × period are >0.50.

[5]De novo = milk FA <16 carbons in length; mixed = milk FA 16 carbons in length; preformed = milk FA >16 carbons in length.

regression after 2 rounds of removing outliers in the data. This method is more accurate than measuring BW at the beginning and end of each treatment period. However, a question still exists whether this BW gain was a gain of body tissue or a gain of digesta. Perhaps SBO induced cholecystokinin, decreased rumen motility, increased ruminal contents mass (Della-Fera and Baile, 1980), and consequently increased BW without altering body fat mass, as observed in a previous work supplementing UFA (Bradford et al., 2008). However, we did not observe any abrupt BW change when cows switched treatments. In addition, DMI and DM digestibility were similar for SBO and PAT. Therefore, it seems unlikely that the BW gain in SBO was caused by increased mass of digesta. The order of fat deposition in cattle is internal fat, subcutaneous fat, and then inter- and intramuscular fat (Allen, 1976). Thus, it may be that the increase in t10c12CLA and slight increase in insulin in our current study were sufficient to affect the deposition of internal fat, but not subcutaneous and muscular fat, and therefore not BCS. Further examination is required.

In the current study, both PAT and SBO cows had MFD, with milk fat being 3.07% in PAT cows and 2.42% in SBO cows. Reduced milk fat yield in SBO in our current study, compared with PAT, was entirely attributed to de novo FA synthesis. A reduction in de novo FA yield is typical in diet-induced MFD (Boerman et al., 2015). Baumgard et al. (2001) showed that infusion of t10c12CLA resulted in greater MFD and proportionally greater reduction in de novo FA than preformed FA. As milk fat % was lower in SBO compared with PAT (2.42 vs. 3.07%), the reduction of de novo FA in SBO was fully expected. In contrast to the MFD study where both high starch and high oil were used (Loor et al., 2005), we observed no change in the yield of preformed FA and a 14% increase in plasma insulin concentration. Blood insulin concentration is generally negatively associated with yield of milk preformed FA during MFD (Corl et al., 2006; Winkelman and Overton, 2013). Therefore, glucogenic-insulin theory likely does not explain the MFD in our current study. Whether the 14% increase in insulin had any effect on adipose depots is not clear. Compared with PAT, SBO increased amount of absorbed FA per day and blood triglyceride concentrations. Based on intake and digestibility data, SBO cows digested and absorbed 183 g/d more 18-carbon FA (Table 4) than PAT cows. Perhaps this increase in absorbed C18 FA prevented the normal drop of preformed milk FA normally seen during MFD.

We suggest that part, if not all, of the energy partitioning in the current study was mediated by t10c12CLA and insulin, considering that the effects of t10c12CLA and insulin on energy partitioning are well established. A meta-analysis by Harvatine et al. (2009) showed that milk energy output significantly decreased in cows suffering CLA-induced MFD and t10c12CLA infusion significantly increased expression of lipogenic genes in adipose tissues, which suggested that t10c12CLA favored energy flow toward body tissue gain instead of milk synthesis. Based on previous work (Vernon, 2005; Bauman et al., 2011), insulin also favors energy partitioning toward body tissue gain, by inhibition of lipolysis and stimulation of lipid synthesis in adipose tissues. Al-Trad et al. (2009) observed that glucose infusion, which likely increased insulin concentration, increased BW and back fat linearly but did not alter milk energy output. These results support our speculation that insulin could play a role in the current study in terms of energy partitioning between milk fat and adipose tissue, although the effect of insulin on milk energy output is minor compared with that of t10c12CLA. To better understand the individual roles of t10c12CLA and insulin on MFD and nutrient partitioning (Table 7), we compared results in our current study with those from Boerman et al. (2015). Boerman et al. (2015) induced MFD by feeding high starch, whereas we fed high UFA while keeping dietary starch content similar. The high-starch diet of Boerman et al. (2015) increased *trans*-10 C18:1 yield in milk by 44%, whereas SBO in our study increased *trans*-10 C18:1 yield in milk by 56%. However, high starch caused no change in t10c12CLA, whereas SBO increased t10c12CLA yield by 69%. Along with this, high starch increased insulin concentration 33%, but SBO in the current study increased insulin only 14%. The 14% increase of insulin in our current study is noteworthy because insulin concentration was not expected to be different between diets containing similar starch contents, as in PAT and SBO. Perhaps, the increase of insulin in SBO was due to the increased supply of UFA in SBO. Bradford et al. (2008) demonstrated that UFA increased plasma glucagon-like peptide 1, and Shigeto et al. (2015) showed that glucagon-like peptide 1 stimulated the secretion of insulin. However, to our knowledge, no experiment directly studied this pathway; this speculation needs further examination. The increase in insulin secretion might also be induced by SBO increasing rumen production of t10c12CLA. As less energy was used for milk fat synthesis in the MFD cows, more sparing of energy would induce the secretion of insulin. However, Harvatine et al. (2009) observed that increased insulin was not induced by t10c12CLA infusions in CLA-induced MFD studies. As Harvatine et al. (2009) only infused t10c12CLA for 4 d, perhaps

Table 7. Comparison of results between Boerman et al. (2015) and the current study

Item	Boerman et al. (2015)[1] HS vs. HFF (*P*-value)	Current study[2] SBO vs. PAT (*P*-value)
Insulin, μg/L	33% (<0.01)	14% (0.03)
trans-10 C18:1, g/d	44% (<0.01)	56% (<0.01)
trans-10,*cis*-12 C18:2, g/d	0% (0.17)[3]	69% (<0.01)
DMI, kg/d	2% (0.10)	−0.4% (0.65)
NE_L, Mcal/kg	0.5% (0.64)	1% (0.51)
Milk yield, kg/d	3% (0.02)	0.9% (0.58)
Milk fat, kg/d	−7% (<0.01)	−18% (<0.01)
De novo FA, g/d	16% (<0.01)	−22% (<0.01)
Mixed FA, g/d	−25% (<0.01)	−36% (<0.01)
Preformed FA, g/d	0.5% (0.78)	4% (0.27)
BW gain, kg/d	136% (<0.01)	142% (0.04)
BCS gain, point/28 d	250% (<0.01)	9% (0.81)
MilkE,[4] Mcal/d	−1% (0.05)	−7% (<0.01)
ΔBodyE,[5] Mcal/d	151% (<0.01)	138% (<0.01)

[1]Treatments were either a high fiber and fat (HFF) diet containing a 2.5% (DM basis) palmitic acid–enriched fatty acid (FA) supplement or a high-starch diet (HS) diet containing a mixture of dry ground and high-moisture corn. The results indicate the values for HS relative to those for HFF expressed as a percentage.

[2]Treatments were diets containing either 2.5% (DM basis) palmitic acid–enriched triglyceride (PAT) or 2.5% (DM basis) soybean oil (SBO). The results indicate the values for SBO relative to those for PAT expressed as a percentage.

[3]The actual value of *trans*-10,*cis*-12 C18:2 in HFF was not provided in Boerman et al. (2015).

[4]MilkE is net energy used for milk synthesis.

[5]ΔBodyE is net energy captured in body tissue.

long-term studies with t10c12CLA-infusion are needed to examine the relationship between t10c12CLA and insulin. Despite the considerable differences in insulin and t10c12CLA responses across the 2 studies (Boerman et al., 2015, and the current study), the depression in milk fat and gain in body reserves were similar (Table 7). We suggest that t10c12CLA and insulin are both critical for the change in energy partitioning that occurs during MFD, and they can function relatively independent of each other.

In our current study, we fed 2.5% supplemental fat and observed no significant difference in DMI between PAT and SBO. These results agree with the studies of Avila et al. (2000) and Kargar et al. (2010), who found that feeding unsaturated fat at 2% of the diet did not alter DMI. In contrast, several others have shown that feeding cows with diets containing unsaturated fat depressed DMI (Pantoja et al., 1996; Harvatine and Allen, 2006; Bradford et al., 2008). Avila et al. (2000) proposed that 2% unsaturated fat would not alter the cellulolytic bacteria community and therefore not alter DMI. In support of Avila et al. (2000), we found no difference in the digestibility of DM. The NDF digestibility in the current study was lower than expected, perhaps because our diets had high starch content, which is known to depress NDF digestibility.

Similar to the DMI results, yields of lactose and milk were not different in our study. In contrast, Boerman et al. (2015) observed higher milk yield in a high-starch diet that caused MFD. As glucose is an important substrate for supporting the synthesis of de novo FA, depressed de novo FA synthesis in MFD should increase glucose availability for lactose synthesis, with a concomitant increase in milk yield (Boerman and Lock, 2014). However, in our current study, we did not observe an increase in milk yield. We suggest that the increased blood insulin and the small increase in available glucose from reduced de novo FA synthesis were used to support the extra protein synthesis we observed; this is consistent with previous work showing that insulin stimulated milk protein synthesis in Griinari et al. (1997) and Bionaz et al. (2012). As observed previously in the MFD studies supplementing unsaturated fat and FA (Firkins and Eastridge, 1994; Pantoja et al., 1996; Relling and Reynolds, 2007), SBO decreased milk fat yield. However, milk fat content was lower than expected, even in the PAT diet. Reasons for the low fat content in our study are not clear, but the 20% drop in milk fat from PAT to SBO was still within a reasonable range for MFD, and was less than the maximal drop proposed by Griinari and Bauman (2006). In our study, we formulated basal diets that put cows at high risk of MFD by increasing starch level to 32% and lowering NDF to 25%. No health issues or metabolic disorders were detected during the study. Thus, the low milk fat percentage and milk fat yield in both treatment groups likely were the results of our high-starch, low-NDF basal diet (Griinari and Bauman, 2006). In the SBO group, milk fat content dropped to 2.4%, which likely was the result of adding unsaturated fat to a high-starch, low-NDF diet.

CONCLUSIONS

Feeding diets with similar levels of fat, starch, fiber, and protein, but containing soybean oil instead of palmitic acid–enriched triglycerides, caused MFD and increased BW gain without altering milk yield. Reduced milk fat yield was due to decreased de novo FA synthesis. The diet with soybean oil also decreased ECM and 3.5% FCM but increased milk protein concentration and yield. The diet with soybean oil increased concentration and yield of *trans*-10 C18:1 and t10c12CLA in milk, which likely explains most of the observed MFD. The diet with soybean oil also increased plasma insulin concentration. With similar dietary starch content, the

diet containing soybean oil partitioned 5.0 percentage units more energy toward body tissue gain and 4.7 percentage units less energy toward milk synthesis, compared with the diet containing palmitic acid–enriched triglycerides. We suggest that the significant increase of t10c12CLA, instead of insulin, resulted in the observed changes in BW gain and energy partitioning in the current study; however, this hypothesis needs to be directly examined in future studies.

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