



Altering the ratio of dietary palmitic and oleic acids affects production responses during the immediate postpartum and carryover periods in dairy cows

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ABSTRACT

The objectives of our study were to determine the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids on production and metabolic responses of early-lactation dairy cows during the immediate postpartum period and to evaluate carryover effects of the treatment diets early in lactation. Fifty-six multiparous cows were used in a randomized complete block design and randomly assigned to 1 of 4 treatments (14 cows per treatment) fed from 1 to 24 d in milk (DIM). The treatments were: (1) control (CON) diet not supplemented with fatty acids (FA); (2) diet supplemented with a FA blend containing 80% C16:0 and 10% *cis*-9 C18:1 (80:10); (3) diet supplemented with a FA blend containing 70% C16:0 and 20% *cis*-9 C18:1 (70:20); and (4) diet supplemented with a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1 (60:30). The FA supplement blends were added at 1.5% of diet DM by replacing soyhulls in the CON diet. All cows were offered a common diet from d 25 to 63 postpartum (carryover period) to evaluate carryover effects. Three preplanned contrasts were used to compare treatment differences: CON versus FA-supplemented diets (80:10 + 70:20 + 60:30)/3; the linear effect of *cis*-9 C18:1 inclusion in diets; and the quadratic effect of *cis*-9 C18:1 inclusion in diets. During the treatment period, FA-supplemented diets increased milk yield, 3.5% fat-corrected milk (FCM), and energy-corrected milk (ECM) compared with CON. Compared with CON, FA-supplemented diets increased milk fat content, milk fat yield, yield of mixed FA, and tended to increase protein yield and lactose yield. Also, compared with CON, FA-supplemented diets tended to increase body condition score (BCS) change. A treat-

ment by time interaction was observed for body weight (BW), due to 80:10 inducing a greater BW loss over time compared with other treatments. Increasing *cis*-9 C18:1 in FA treatments tended to linearly increase dry matter intake (DMI) but did not affect milk yield, 3.5% FCM, ECM, and the yields of milk fat, protein and lactose. Increasing *cis*-9 C18:1 in FA treatments linearly decreased milk fat content and milk lactose content. Also, increasing *cis*-9 C18:1 in FA treatments linearly decreased BW and BCS losses. During the carryover period, compared with CON, FA-supplemented diets tended to increase milk yield. Also, FA-supplemented diets increased 3.5% FCM, ECM, and milk fat yield, and tended to increase milk protein yield compared with CON. A treatment by time interaction was observed for BW due to 80:10 increasing BW over time compared with CON. Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased milk yield and ECM compared with a nonfat supplemented control diet. Increasing *cis*-9 C18:1 in the FA supplement increased DMI and reduced BW and BCS losses. Additionally, the fat-supplemented diets fed during the immediate postpartum period had a positive carryover effect during early lactation, when cows were fed a common diet.

Key words: palmitic acid, oleic acid, early lactation, carryover

INTRODUCTION

During the immediate postpartum period (3 to 4 wk following parturition), high-producing cows are challenged with large metabolic demands due to the sudden increase in energy requirements that cannot be met by feed intake alone (van Knegsel et al., 2007). During this stage, dairy cows enter a state of negative energy balance, leading to an increase in mobilization of adipose tissue and release of nonesterified fatty acids (NEFA) into circulation to be metabolized by the liver and other tissues and incorporated into milk fat in the mammary gland (Drackley, 1999). Intensive

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body reserve mobilization and the resulting elevated plasma NEFA concentrations can lead to alterations in immune function and increase the risk and severity of both metabolic and infectious diseases (Sordillo et al., 2009; Sordillo, 2016). Higher energy intake during the immediate postpartum results in lower circulating NEFA (Rabelo et al., 2005) and has been associated with improved health (Esposito et al., 2014) and performance (Rabelo et al., 2003). Approaches to increase energy density of the diet and energy intake of postpartum cows include increasing dietary starch content and supplementing fatty acids (FA; McCarthy et al., 2015; Piantoni et al., 2015a). Feeding high starch diets or more fermentable starch sources that promote greater ruminal propionate production during early lactation could be hypophagic and therefore further reduce DMI and increase the risk of ruminal acidosis and displaced abomasum (Allen and Piantoni, 2013; Albornoz and Allen, 2018). Inconsistent production and metabolic responses to FA supplementation in early-lactation cows have been observed, which are likely associated with the FA profile of the supplements, timing and level of supplementation, and interactions with other dietary and animal factors (e.g., Greco et al., 2015; Piantoni et al., 2015a; de Souza and Lock, 2019). Hence, determining dairy cow responses to specific FA or combination of FA is of particular importance.

To our knowledge, few studies were designed to evaluate the effects of different FA ratios on production responses of dairy cows. Greco et al. (2015) observed that decreasing the ratio of omega-6 to omega-3 FA in the diet of lactating dairy cows while maintaining similar dietary concentrations of total FA improved productive performance in early lactation (from 14 to 105 DIM). A dietary omega-6 to omega-3 ratio of approximately 4:1 increased DMI and the yield of milk and milk components compared with a 6:1 ratio. Additionally, the authors reported that not all production responses could be accounted for by differences in nutrient intake, which suggests that altering the dietary ratio of FA can influence nutrient partitioning to favor an increased proportion of the total net energy consumed allocated to milk synthesis. We recently observed that feeding a C16:0 supplement during early lactation increased the yield of ECM, but increased BW loss and plasma NEFA concentration when the supplement was fed in the first 24-d of lactation (de Souza et al., 2019a; de Souza and Lock, 2019). In postpeak cows, we observed that feeding a FA blend with a high content of C16:0 (80% C16:0) increased milk energy output, whereas feeding a FA blend with a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1) increased plasma insulin and BW gain compared with nonfat supplemented control diets (de Souza et al., 2018).

Similarly, de Souza et al. (2019b) evaluated 4 different dietary ratios of C16:0 (from 80 to 60%) and *cis*-9 C18:1 (from 10 to 30%) in supplemental fat blends to postpeak cows and reported that increasing *cis*-9 C18:1 increased BW gain and plasma insulin.

We hypothesized that increasing the amount of C16:0 in a FA supplement would increase milk energy output due to differences in milk fat yield responses while increasing *cis*-9 C18:1 would reduce body reserves mobilization in early lactation. We also postulated that feeding FA supplements in the immediate postpartum would result in positive carryover effects on performance during early lactation. Therefore, our objectives were to determine the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on production and metabolic responses of early-lactation dairy cows during the immediate postpartum period and to evaluate carryover effects of the treatment diets in early-lactation.

MATERIALS AND METHODS

Animal Housing and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). The experiment began on February 22, 2017, and finished on September 15, 2017. Cows were fed once daily (0900 h) at 120% of expected intake during the treatment and carryover periods and milked twice daily (0400 and 1430 h). Standard reproduction and health herd checks and breeding practices were maintained during this study. This article reports the effect of these diets on DMI, yield of milk and milk components, BW, BCS, and milk FA profile. The companion paper (de Souza et al., 2021) describes treatment effects on nutrient digestibility, energy intake and balance, and plasma metabolites and hormones.

Design and Treatment Diets

Fifty-six multiparous Holstein cows at the Michigan State University Dairy Cattle Teaching and Research Center were used in a randomized complete block design. Cows were blocked into 14 blocks by BCS observed ~30 d before expected parturition date (up to 0.50-unit difference using the 1 = thin, 5 = fat scale in 0.25 increments), previous lactation 305-d mature-equivalent milk yield (within 2,000 kg), parity (up to 1 lactation difference), and calving date (up to 30 d). Cows within each block were randomly assigned to 1 of 4 treatments fed from 1 to 24 DIM. Each cow was housed in the same tiestall, assigned by parturition order, throughout the entire period. The treatments were combinations of 2

commercially available FA supplements that differed in FA profile, which were blended to achieve different ratios of C16:0 and *cis*-9 C18:1 in the FA treatment blends (Table 1). The treatments were: (1) control (**CON**) diet not supplemented with FA; (2) diet supplemented with a FA blend containing 80% C16:0 and 10% *cis*-9 C18:1 (**80:10**); (3) diet supplemented with a FA blend containing 70% C16:0 and 20% *cis*-9 C18:1 (**70:20**); and (4) diet supplemented with a FA blend containing 60% C16:0 and 30% *cis*-9 C18:1 (**60:30**). The FA supplement blends were added at 1.5% of diet DM by replacing soyhulls in the CON diet. Treatment diets were mixed daily in a tumble-mixer, and treatment commenced the morning following parturition. From 25 to 63 d postpartum (carryover period), all cows were offered a common diet, mixed daily in a mixer wagon. The ingredient and nutrient composition of the diets fed as TMR, including the close-up ration for reference, are described in Table 2. All rations were formulated to meet or exceed cows predicted requirements for minerals and vitamins according to NRC (2001).

Data and Sample Collection

All samples and body measurements were collected or recorded on the same day of the week during the entire experiment, so all collection days are ± 3 d. Milk yield and feed offered and refused were recorded daily throughout the experiment. Samples of all diet ingredients (0.5 kg) and orts from each cow ($\sim 12.5\%$) were collected weekly during the experiment and stored in plastic bags at -20°C until processed. Milk sam-

ples were collected twice a week at each milking and stored with preservative at 4°C for component analysis (Bronopol tablet; D&F Control Systems, San Ramon, CA). An additional milk sample was collected at each milking on d 5, 12, 19, and 35 postpartum and stored without preservative at -20°C for determination of FA profile. Body weight was recorded weekly prepartum and 3 times per week postpartum. Body condition was scored weekly by 3 trained investigators on a 5-point scale as described by Wildman et al. (1982).

Sample Analysis

Feed and orts samples were dried in a 55°C forced-air oven for 72 h, and DM content was calculated. Before drying, feed ingredients were composited monthly. Orts from individual cows were dried to calculate DMI weekly, but only orts collected on d 5, 12, and 19 postpartum were processed further and analyzed for nutrient composition. Once dried, samples of feed ingredients and orts were ground in a Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA) and analyzed for ash, NDF, indigestible NDF, CP, starch, and FA concentration as described by Boerman et al. (2017).

Milk samples were analyzed for fat, true protein, and lactose concentrations by mid-infrared spectroscopy (AOAC, 1990; method 972.160) (NorthStar Michigan Lab, Grand Ledge, MI). Yields of 3.5% FCM, ECM, milk energy, and milk components were calculated using milk yield and component concentrations from each milking, summed for a daily total, and averaged for each week. Milk samples stored without preservative

Table 1. Proportion of each fatty acid (FA) supplement for treatment blends and FA profile of FA blends

Item	Treatment ¹		
	80:10	70:20	60:30
% of each FA supplement in treatment blends			
Palmitic acid-enriched FA supplement ²	91.0	62.0	33.0
Ca salts of palm FA supplement ³	9.0	38.0	67.0
FA profile of each FA blend, g/100 g of FA			
C14:0	0.67	0.78	0.88
C16:0	81.2	70.7	59.7
C18:0	1.82	1.91	2.00
<i>cis</i> -9 C18:1	10.8	20.0	29.7
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.95	4.72	6.55
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.11	0.17	0.23

¹Treatments were: 80:10 (1.5% of FA supplement blend to provide $\sim 80\%$ C16:0 and 10% *cis*-9 C18:1); 70:20 (1.5% of FA supplement blend to provide $\sim 70\%$ C16:0 and 20% *cis*-9 C18:1); 60:30 (1.5% of FA supplement blend to provide $\sim 60\%$ C16:0 and 30% *cis*-9 C18:1).

²Palmitic acid-enriched FA supplement (Nutracor; Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.64 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.88 of *cis*-9 C18:1, and 99.0% total fatty acids.

³Ca salts of palm FA supplement (Nutracal; Wawasan Agrolipids). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.0 of C16:0, 2.10 of C18:0, 39.8 of *cis*-9 C18:1, and 83.2% total fatty acids.

were composited by milk fat yield and centrifuged at $17,800 \times g$ for 30 min at 4°C to collect the fat cake. Milk lipids were extracted, and FA-methyl esters prepared and quantified using GLC according to Lock et al. (2013). Yield of individual FA (g/d) in milk fat were calculated by using milk fat yield and FA concentrations to determine yield on a mass basis using the molecular

weight of each FA while correcting for glycerol content and other milk lipid classes (Piantoni et al., 2013).

Statistical Analysis

Data were analyzed separately for the treatment (1–24 d postpartum) and carryover (25–63 d postpartum)

Table 2. Ingredient and nutrient composition of close-up diet, treatment diets, and carryover diet

Item	Diet					
	Close-up	Treatment ¹				Carryover
		CON	80:10	70:20	60:30	
Ingredient, % of DM						
Corn silage	42.0	29.7	29.7	29.7	29.7	26.5
Alfalfa silage	—	10.9	10.9	10.9	10.9	13.8
Alfalfa hay	—	12.4	12.4	12.4	12.4	—
Grass hay	35.5	—	—	—	—	—
Wheat straw	—	—	—	—	—	2.65
Ground corn	7.09	19.6	19.6	19.6	19.6	14.7
High moisture corn	—	5.03	5.03	5.03	5.03	16.1
Soybean meal	8.11	13.9	13.9	13.9	13.9	13.9
Soyhulls	—	3.10	1.55	1.50	1.45	3.00
SoyChlor ²	2.52	—	—	—	—	—
Whole cottonseed	—	—	—	—	—	4.66
Protein supplement ³	1.13	1.42	1.42	1.42	1.42	1.19
C16:0-enriched FA supplement ⁴	—	0.00	1.26	0.87	0.48	—
Ca salts of palm FA supplement ⁵	—	0.00	0.29	0.73	1.16	—
Mineral and vitamin mix ⁶	2.60	3.95	3.95	3.95	3.95	3.52
Nutrient composition, % of DM						
NDF	38.5	30.7	29.5	29.5	29.4	28.8
Forage NDF	34.9	23.0	23.0	23.0	23.0	20.3
CP	14.6	16.9	16.7	16.7	16.7	16.9
Starch	17.2	24.6	24.6	24.6	24.6	27.6
NE _L , ⁷ Mcal/kg of DM	—	1.57	1.63	1.64	1.62	—
FA	1.82	2.49	4.01	4.01	3.99	2.94
16:0	0.28	0.36	1.57	1.43	1.26	0.30
18:0	0.06	0.07	0.10	0.10	0.10	0.09
<i>cis</i> -9 18:1	0.29	0.46	0.64	0.77	0.90	0.40
<i>cis</i> -9, <i>cis</i> -12 18:2	0.82	1.22	1.26	1.28	1.31	1.00
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.17	0.15	0.15	0.15	0.15	0.15

¹Control (CON) = diet not supplemented with fatty acid (FA); 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1 (80:10); 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis*-9 C18:1 (70:20); 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

²West Central Soy, Ralston, IA.

³Spectrum Agribiue (Perdue Agribusiness, Salisbury, MD). The supplement contained (% of DM) 89.5 CP, 55.6 total essential amino acids, 11.0 leucine, 8.0 valine, 7.8 lysine, 6.2 phenylalanine, 5.4 histidine, 5.2 methionine.

⁴Nutracor (Wawasan Agrolipids, Johor, Malaysia). The supplement contained (g/100 g of fatty acid) 0.64 of C14:0, 84.5 of C16:0, 1.80 of C18:0, 7.88 of *cis*-9 C18:1, and 99.0% total fatty acids.

⁵Nutractal (Wawasan Agrolipids). The supplement contained (g/100 g of fatty acid) 1.0 of C14:0, 48.0 of C16:0, 2.10 of C18:0, 39.8 of *cis*-9 C18:1, and 83.2% total fatty acids.

⁶Vitamin-mineral mix for the close-up diet contained (DM basis): 54.8% SoyChlor, 13.9% limestone, 10.0% rumen-protected choline, 8.8% dicalcium phosphate, 4.2% magnesium sulfate, 1.8% salt, 1.8% yeast, 4.4% trace minerals and vitamins, and 0.3% selenium yeast 600 (600 mg of Se/kg). Vitamin-mineral mix for the treatment diets contained (DM basis): 27.9% molasses, 15.3% limestone, 12.2% sodium bicarbonate, 11.8% blood meal, 8.7% dicalcium phosphate, 6.1% trace minerals and vitamins, 5.7% rumen-protected choline, 4.4% magnesium sulfate, 3.9% salt, 2.7% animal fat, 0.9% yeast, and 0.4% selenium yeast 600 (600 mg of Se/kg). Vitamin-mineral mix for the carryover diet contained (DM basis): 30.1% limestone, 25.3% sodium bicarbonate, 10.1% salt, 7.1% urea, 6% potassium chloride, 6% dicalcium phosphate, 5.7% animal fat, 5.7% magnesium sulfate, 3.9% trace minerals and vitamins, and 0.2% selenium yeast 600 (600 mg of Se/kg).

⁷Calculated using nutrient digestibility values as presented in de Souza et al. (2021).

periods as a complete block design. Cow was considered the experimental unit (14 cows per treatment and 14 blocks). All weekly data were analyzed using the MIXED procedure of SAS v.9.2 (SAS Institute, Inc. Cary, NC) with week being the repeated measurement.

The model used included:

$$Y_{ijkl} = \mu + B_i + C(B_i F_k)_j + F_k + T_1 + F_k T_1 + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ = overall mean, B_i = random effect of block, $C(B_i F_k)_j$ = random effect of cow within block and treatment diet, F_k = fixed effect of treatment during the treatment period, T_1 = fixed effect of time, $F_k T_1$ = fixed effect of treatment by time interaction, and e_{ijkl} = residual error. In our preliminary model, we included Julian date of parturition as a factor in the model, but this factor was deemed not significant for all variables and removed from the final model.

The first-order autoregressive covariate structure was used for repeated measures analysis due to the lowest resulting Bayesian information criterion for the majority of variables measured. For milk FA analysis during the carryover period only one sample was taken and a reduced model without the effect of time was used. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Significance was declared at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Tendencies were declared at $P \leq 0.10$ for main effects and $P \leq 0.15$ for interactions. Three preplanned contrasts were used to compare treatment differences: CON versus FA-supplemented diets, (80:10 + 70:20 + 60:30)/3; the linear effect of *cis*-9 C18:1 inclusion in diets, and the quadratic effect of *cis*-9 C18:1 inclusion in diets. All cows were in apparent good health at the beginning of the study and treatment groups were not different with regard to 305-d mature-equivalent milk production ($P = 0.84$; 13,368 \pm 1,546 kg), BW ($P = 0.44$; 753 \pm 83), or BCS ($P = 0.93$; 3.70 \pm 0.35) prepartum.

RESULTS

Diets and Nutrient Composition, and Health Incidents

All cows received a common close-up diet before parturition (Table 2). During the treatment period, the CON diet contained (DM basis) 30.7% NDF, 23.0% forage NDF, 24.6% starch, and 2.49% total FA. As expected, the 80:10 treatment mainly increased dietary C16:0, whereas 70:20 and 60:30 increased both C16:0 and *cis*-9 C18:1, compared with CON. During the

Table 3. Health incidents during the experiment within treatment diet¹

Item	Treatment ²			
	CON	80:10	70:20	60:30
During treatment period				
Ketosis	2	3	2	2
Metritis	1	—	—	—
Milk fever	1	1	1	—
Retained placenta	3	1	1	3
Displaced abomasum	1	—	—	—
During carryover period				
Lame	1	—	—	—
Mastitis	—	2	—	1

¹Retained placenta was defined as a failure to expel fetal membranes within 24 h after calving. Metritis was defined as the presence of fetid, watery, red-brown uterine discharge, and body temperature greater than 39.5°C. Clinical ketosis was recognized by clinical symptoms as anorexia and reduced milk production, accompanied by ketone bodies concentrations above 10 mg/dL in urine (Ketostix Reagent Strips, Bayer AG, Leverkusen, Germany). Milk fever was recognized by showing muscle weakness, nervousness, muscle shaking, cold ears, and inability to rise.

²Control (CON) = diet not supplemented with fatty acid (FA); 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1 (80:10); 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis*-9 C18:1 (70:20); 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

carryover period, diets were adjusted to reduce forage and increase starch content and starch fermentability. Therefore, the carryover diet contained (DM basis) 28.8% NDF, 20.3% forage NDF, 27.6% starch, and 2.94% total FA.

This study was not designed to evaluate treatment effects on health incidents. Therefore, only a summary of health incidents is presented in Table 3. Ketosis was the primary health incident observed with 2, 3, 2 and 2 cases for CON, 80:10, 70:20, and 60:30, respectively. Also, we observed 3, 1, 1, and 3 cases of retained placenta for CON, 80:10, 70:20, and 60:30, respectively. The primary health incident during the carryover period was mastitis.

Production Responses During the Treatment Period

The FA-supplemented diets increased milk yield ($P = 0.05$; Table 4), 3.5% FCM ($P < 0.01$), and ECM ($P = 0.01$) compared with CON. Compared with CON, FA-supplemented diets increased milk fat content ($P = 0.03$), milk fat yield ($P < 0.01$), and tended to increase protein yield ($P = 0.06$). We did not observe treatment differences for milk protein content ($P = 0.21$), milk lactose content ($P = 0.26$), or milk lactose yield ($P = 0.11$).

Increasing *cis*-9 C18:1 in FA treatments linearly increased DMI ($P = 0.03$; Table 4). However, altering *cis*-9 C18:1 in FA treatments did not affect milk yield,

3.5% FCM, ECM, and the yields of milk protein and lactose (all $P > 0.10$). Increasing *cis-9* C18:1 in FA treatments tended to quadratically decrease milk fat content ($P = 0.10$), milk fat yield ($P = 0.08$), and milk lactose content ($P = 0.05$). Also, increasing *cis-9* C18:1 in FA treatments linearly decreased BW ($P = 0.02$) and BCS ($P = 0.04$) losses, and tended to increase BW ($P = 0.10$).

The increase in DMI, milk yield, and ECM was consistent over time for all treatments (Figure 1). A treatment by time interaction was observed for BW ($P < 0.01$), due to 80:10 inducing a greater BW loss over time compared with other treatments (Figure 2).

Production Responses During the Carryover Period

Compared with CON, FA-supplemented diets tended to increase milk yield ($P = 0.08$; Table 5). Additionally, FA-supplemented diets increased 3.5% FCM ($P = 0.02$), ECM ($P = 0.02$), and milk fat yield ($P = 0.02$), and tended to increase milk protein yield ($P = 0.10$) compared with CON. Compared with CON, FA-supplemented diets consistently increased milk yield and ECM over time peaking at wk 5 (Figure 1). Although FA-supplemented diets increased milk lactose content ($P < 0.01$), we did not observe treatment differences for milk fat ($P = 0.19$) or milk protein content ($P =$

0.65). Compared with CON, FA-supplemented diets decreased BCS ($P = 0.02$).

Increasing *cis-9* C18:1 in FA treatments linearly increased milk fat yield ($P = 0.01$; Table 5), 3.5% FCM ($P = 0.02$) and ECM ($P = 0.02$). There was no effect of increasing *cis-9* C18:1 in FA treatments on other production variables or BW during the carryover period (all $P > 0.10$).

Although we did not observe treatment differences for DMI ($P > 0.10$), DMI increased over time, peaking at wk 6 for all treatments (Figure 1). A treatment by time interaction was observed for BW ($P = 0.10$), due to 80:10 increasing BW over time compared with CON (Figure 2).

Milk FA Concentration and Yield During the Treatment Period

Milk FA are derived from 2 sources: <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis-9* C16:1) originate from both de novo synthesis in the mammary gland and extraction from plasma. Compared with CON, FA-supplemented diets increased concentration of mixed ($P < 0.01$; Table 6) but did not affect the concentration of de novo ($P = 0.29$) and preformed FA ($P = 0.16$). The

Table 4. Milk production, milk composition, BW, and BCS for cows fed treatment diets during the fresh period (d 1 to 24 postpartum)

Item ¹	Treatment ²				SEM	Contrast ³			P-value ⁴	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt × Time
DMI, kg	20.3	20.7	20.9	21.8	0.48	0.14	0.03	0.51	<0.01	0.97
Milk yield, kg/d										
Milk	46.5	48.6	48.8	49.7	1.39	0.05	0.14	0.42	<0.01	0.84
3.5% FCM	50.1	54.8	54.1	54.7	1.27	<0.01	0.74	0.97	<0.01	0.52
ECM	50.2	54.8	53.5	54.3	1.18	0.01	0.41	0.71	0.04	0.50
Milk composition										
Fat, kg/d	1.90	2.15	2.08	2.09	0.06	<0.01	0.06	0.08	0.03	0.27
Fat, %	4.06	4.45	4.26	4.21	0.12	0.03	0.32	0.10	<0.01	0.51
Protein, kg/d	1.41	1.56	1.50	1.52	0.05	0.06	0.25	0.20	0.83	0.63
Protein, %	3.13	3.25	3.19	3.22	0.06	0.21	0.42	0.53	<0.01	0.64
Lactose, kg/d	2.11	2.34	2.25	2.25	0.09	0.11	0.42	0.21	<0.01	0.55
Lactose, %	4.80	4.88	4.82	4.80	0.03	0.26	0.56	0.05	<0.01	0.82
BW, kg	693	678	705	715	16.1	0.71	0.10	0.69	<0.01	<0.01
BW change, kg/d	-1.55	-2.54	-1.63	-1.48	0.37	0.38	0.02	0.08	NA	NA
BCS	3.46	3.33	3.35	3.38	0.06	0.12	0.40	0.17	<0.01	0.19
BCS change, units/wk	-0.09	-0.14	-0.12	-0.10	0.004	0.09	0.04	0.61	NA	NA

¹3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]; ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

²Control (CON) = diet not supplemented with fatty acid (FA); 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis-9* C18:1; 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis-9* C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis-9* C18:1.

³P-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets, (80:10 + 70:20 + 60:30)/3]; linear and quadratic effects of *cis-9* C18:1 inclusion in supplemental fat.

⁴Trt = treatment. NA = not applicable.

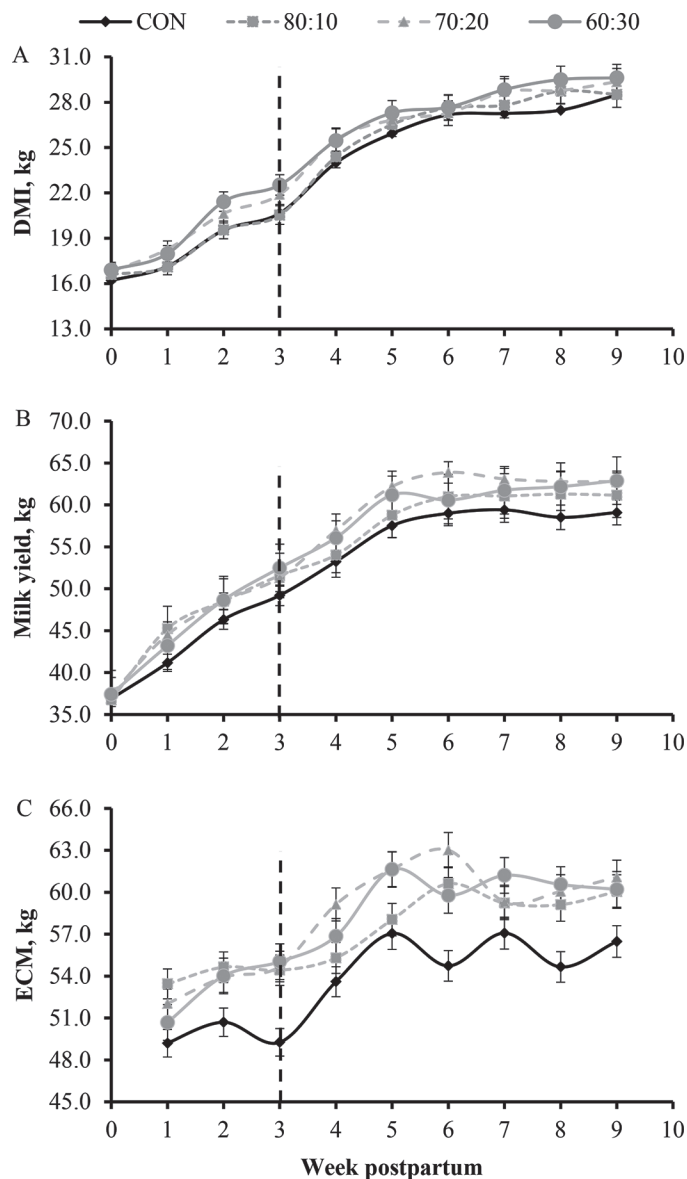


Figure 1. Effects of dietary treatments on DMI (A), milk yield (B), and ECM (C) over time during the treatment (1–24 DIM) and carryover (25–63 DIM) periods. Diets fed during the treatment period included: control (CON) diet not supplemented with fatty acids (FA); diet supplemented with 80% C16:0 + 10% *cis*-9 C18:1 (80:10); diet supplemented with 70% C16:0 + 20% *cis*-9 C18:1 (70:20); and diet supplemented with 60% C16:0 + 30% *cis*-9 C18:1 (60:30). The line on wk 3 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added. During the treatment period, compared with CON, FA-supplemented diets tended to increase DMI ($P = 0.08$), and increased milk yield ($P = 0.05$) and ECM ($P = 0.01$). During the carryover period, compared with CON, FA-supplemented diets tended to increase milk yield ($P = 0.08$) and increased ECM ($P = 0.02$), with no effect on DMI ($P = 0.21$). Among the FA-supplemented diets no differences were observed for these variables ($P > 0.10$). DMI, milk yield, and ECM increased over time in all treatments (all $P < 0.01$). Error bars indicate SEM.

FA-supplemented diets increased the concentration of C16:0 ($P < 0.01$) and *cis*-9 C16:1 ($P < 0.01$) compared with CON (Supplemental Table S1, <https://doi.org/10.3168/jds.2020-19311>). On a yield basis, compared with CON, FA-supplemented diets increased the yield of mixed ($P < 0.01$; Table 6) but did not affect the yield of de novo ($P = 0.93$) and preformed FA ($P = 0.22$).

Increasing *cis*-9 C18:1 in FA treatments quadratically affected mixed FA concentration, with mixed FA being highest in the 80:10 treatment ($P < 0.01$; Table 6). Increasing *cis*-9 C18:1 in FA treatments decreased

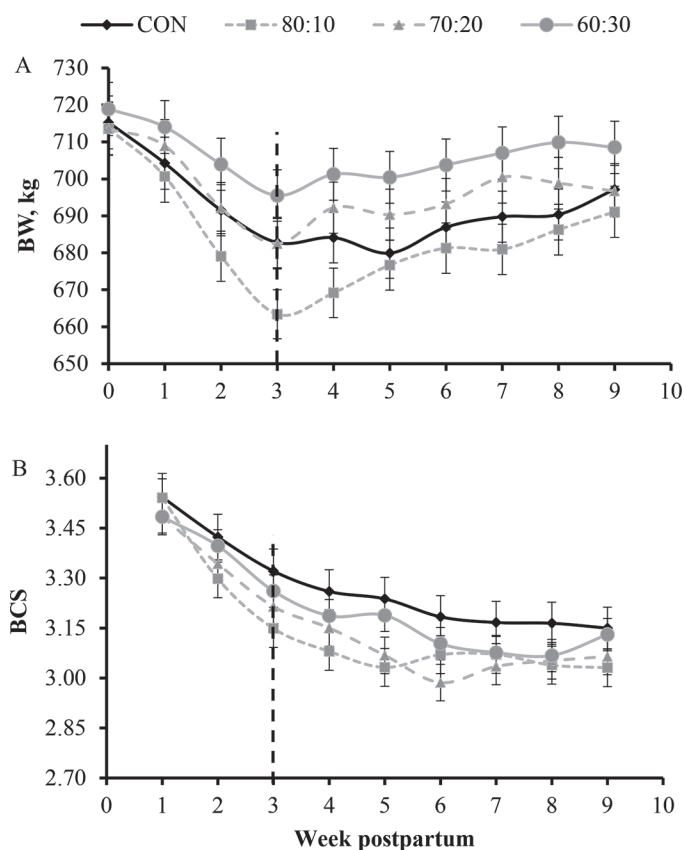


Figure 2. Effects of dietary treatments on BW (A) and BCS (B) over time during the treatment (1–24 DIM) and carryover (25–63 DIM) periods. Diets fed during the treatment period included: control (CON) diet not supplemented with fatty acids (FA); diet supplemented with 80% C16:0 + 10% *cis*-9 C18:1 (80:10); diet supplemented with 70% C16:0 + 20% *cis*-9 C18:1 (70:20); and diet supplemented with 60% C16:0 + 30% *cis*-9 C18:1 (60:30). The line on wk 3 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added. During the treatment period, compared with CON, FA-supplemented diets tended to increase BCS ($P = 0.09$). A treatment by time interaction was observed for BW ($P < 0.01$) due to 80:10 inducing a greater decrease in BW over time compared with other treatments. Also, increasing *cis*-9 C18:1 in FA treatments linearly decreased BW ($P = 0.02$) and BCS ($P = 0.04$) losses, and increasing *cis*-9 C18:1 in FA treatments tended to increase BW ($P = 0.10$). During the carryover period, a treatment by time interaction was observed for BW ($P = 0.10$) due to 80:10 increasing BW over time compared with CON. Error bars indicate SEM.

Table 5. Milk production, milk composition, BW, and BCS for cows fed a common diet during the carryover period (d 25 to 63 postpartum)

Item ¹	Treatment ²					Contrast ³			P-value ⁴	
	CON	80:10	70:20	60:30	SEM	CON vs. FAT	Linear	Quadratic	Time	Trt × Time
DMI, kg	26.7	27.2	27.7	27.9	0.75	0.21	0.14	0.84	<0.01	0.76
Milk yield, kg/d										
Milk	57.8	59.5	60.9	60.8	1.65	0.08	0.46	0.34	<0.01	0.76
3.5% FCM	56.1	59.2	59.8	61.2	1.94	0.02	0.02	0.36	0.030	0.54
ECM	55.6	58.7	59.5	60.3	1.88	0.02	0.02	0.31	<0.01	0.46
Milk composition										
Fat, kg/d	1.91	2.06	2.11	2.13	0.08	0.02	0.01	0.46	0.10	0.82
Fat, %	3.32	3.48	3.38	3.55	0.11	0.19	0.17	0.93	<0.01	0.93
Protein, kg/d	1.68	1.76	1.81	1.77	0.06	0.10	0.17	0.24	<0.01	0.09
Protein, %	2.90	2.96	2.92	2.92	0.06	0.65	0.98	0.61	<0.01	0.40
Lactose, kg/d	2.84	2.97	3.05	3.03	0.11	0.14	0.17	0.44	<0.01	0.07
Lactose, %	4.88	4.98	4.97	4.92	0.03	<0.01	0.19	0.02	0.04	0.07
BW, kg	668	657	676	686	16.3	0.76	0.18	0.80	<0.01	0.10
BW change, kg/d	0.38	0.25	0.32	0.32	0.16	0.63	0.73	0.85	NA	NA
BCS	3.23	3.11	3.06	3.07	0.07	0.02	0.42	0.85	0.61	0.84

¹3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]; energy-corrected milk; ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

²Control (CON) = diet not supplemented with fatty acid (FA); 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

³P-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets: (80:10 + 70:20 + 60:30)/3]; linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

⁴Trt = treatment. NA = not applicable.

C16:0 concentration with the highest concentration in the 80:10 treatment (quadratic, $P < 0.01$; Supplemental Table S1, <https://doi.org/10.3168/jds.2020-19311>). Also, increasing *cis*-9 C18:1 in FA treatments linearly increased concentration of *trans*-6 to 8 and *trans*-9 C18:1 ($P < 0.01$; Supplemental Table S1). Similarly,

increasing *cis*-9 C18:1 in FA treatments quadratically affected mixed FA and C16:0 yield because their yield was highest in the 80:10 treatment ($P < 0.01$; Supplemental Table S2). Increasing *cis*-9 C18:1 in FA treatments linearly increased the yield of C18:0 ($P = 0.03$) and *cis*-9 C18:1 ($P = 0.03$).

Table 6. Summation of milk fatty acid (FA) concentration and yield for cows fed treatment diets during the fresh period (d 1–24 postpartum)

Item ¹	Treatment ²					Contrast ³			P-value ⁴	
	CON	80:10	70:20	60:30	SEM	CON vs. FAT	Linear	Quadratic	Time	Trt × Time
Summation by source, g/100 g of FA										
De novo	18.7	17.7	16.8	18.1	0.98	0.29	0.51	0.22	0.03	0.31
Both	32.3	37.1	35.1	34.9	0.53	<0.01	0.02	<0.01	0.06	0.78
Preformed	49.0	45.2	48.2	47.0	1.34	0.16	0.63	0.32	0.01	0.70
Summation by source, g/d										
De novo	336	332	323	345	24.0	0.93	0.86	0.58	0.02	0.48
Both	579	730	670	662	26.7	<0.01	0.10	<0.01	0.40	0.58
Preformed	830	863	916	894	40.7	0.22	0.15	0.56	0.49	0.43

¹De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Supplemental Tables S1 and S2, respectively (<https://doi.org/10.3168/jds.2020-19311>).

²Control (CON) = diet not supplemented with FA; 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

³P-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets: (80:10 + 70:20 + 60:30)/3]; linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

⁴Trt = treatment.

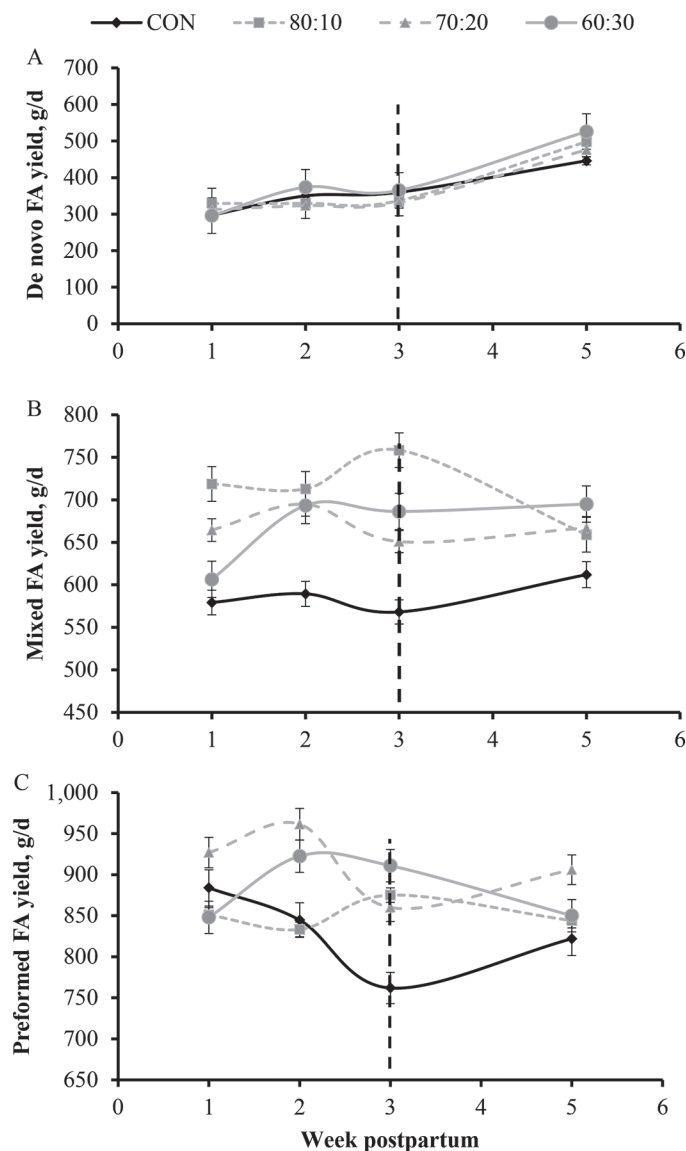


Figure 3. Effects of dietary treatments on the yield of de novo (A), mixed (B) and preformed (C) fatty acids (FA) over time during the treatment (1–24 DIM) and carryover (25–63 DIM) periods. Diets fed during the treatment period included: control (CON) diet not supplemented with FA; diet supplemented with 80% C16:0 + 10% *cis*-9 C18:1 (80:10); diet supplemented with 70% C16:0 + 20% *cis*-9 C18:1 (70:20); and diet supplemented with 60% C16:0 + 30% *cis*-9 C18:1 (60:30). The line on wk 3 indicates the start of the carryover period, when all cows were fed a common diet with no supplemental fat added. During the treatment period, compared with CON, FA-supplemented diets increased yield of mixed ($P < 0.01$) but did not affect the yield of de novo ($P = 0.93$) and preformed FA ($P = 0.22$). Also, increasing *cis*-9 C18:1 in FA treatments quadratically affected mixed FA and C16:0 yield because their yield was highest at 80:10 treatment ($P < 0.01$). During the carryover period, increasing *cis*-9 C18:1 in FA treatments tended to linearly increase the yield of de novo ($P = 0.09$) and mixed FA ($P = 0.08$). Error bars indicate SEM.

The increase in mixed yield was consistent over time for all treatments (Figure 3). Additionally, over time the concentration of preformed FA reduced, whereas de novo FA increased for all treatments.

Milk FA Concentration and Yield During the Carryover Period

Compared with CON, FA-supplemented diets did not affect the concentration of de novo ($P = 0.62$; Table 7), mixed ($P = 0.90$) or preformed FA ($P = 0.73$). Compared with CON, FA-supplemented diets increased the concentration of C18:0 ($P < 0.01$; Supplemental Table S3, <https://doi.org/10.3168/jds.2020-19311>), and decreased concentrations of *trans*-6 to 8 ($P = 0.02$) and *trans*-9 C18:1 ($P = 0.04$). On a yield basis, compared with CON, FA-supplemented diets tended to increase the yield of de novo ($P = 0.09$; Table 7) and mixed ($P = 0.10$) but did not affect preformed FA ($P = 0.37$). Compared with CON, FA-supplemented diets increased the yield of C8:0 ($P = 0.05$; Supplemental Table S4), C18:0 ($P = 0.01$) and tended to increase the yield of C6:0 ($P = 0.07$) and C10:0 ($P = 0.06$).

Increasing *cis*-9 C18:1 in FA treatments did not affect the concentration of de novo, mixed, or preformed FA (all $P > 0.10$; Table 7). Increasing *cis*-9 C18:1 in FA treatments quadratically affected concentrations of C18:0 and *cis*-9 C18:1 because it was highest at 70:20 treatment ($P < 0.05$; Supplemental Table S3, <https://doi.org/10.3168/jds.2020-19311>). On a yield basis, increasing *cis*-9 C18:1 in FA treatments tended to linearly increase the yield of de novo ($P = 0.09$; Table 7) and mixed FA ($P = 0.08$) but did not affect preformed FA yield ($P = 0.38$). Increasing *cis*-9 C18:1 in FA treatments tended to linearly increase the yield of C6:0 ($P = 0.01$; Supplemental Table S4), C8:0 ($P = 0.01$), C10:0 ($P = 0.01$) and C12:0 ($P = 0.03$), and tended to increase the yield of C16:0 ($P = 0.07$).

DISCUSSION

The potential response of supplemental fat during the immediate postpartum period (3 to 4 wk after parturition) and the ideal timing of supplemental fat inclusion are not well described and previous results are inconsistent (e.g., Greco et al., 2015; Piantoni et al., 2015a; de Souza and Lock, 2019). Grummer (1992) suggested based on studies conducted in the early 1990s that supplemental fat had little benefit on cow performance when fed in the first 5 to 7 wk of lactation. In contrast, research is progressing from feeding traditional animal- and plant-fats to more recent interest into the effects of feeding individual FA. Fatty acids C16:0 and *cis*-9 C18:1 are typically the most abundant found

Table 7. Summation of milk fatty acid (FA) concentration and yield for cows fed a common diet during the carryover period (d 25–63 postpartum)

Item ¹	Treatment ²				SEM	Contrast ³		
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic
Summation by source, g/100 g of FA								
De novo	23.8	24.5	22.8	25.4	0.87	0.62	0.37	0.22
Both	32.7	32.3	32.2	33.4	0.63	0.90	0.47	0.18
Preformed	43.6	43.3	45.1	41.2	1.11	0.73	0.27	0.11
Summation by source, g/d								
De novo	446	498	476	526	27.23	0.09	0.09	0.72
Both	612	659	667	695	31.55	0.10	0.08	0.91
Preformed	822	844	906	850	48.38	0.37	0.38	0.50

¹De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originated from extraction from plasma (>16 carbons), and mixed FA originate from both sources (C16:0 plus *cis*-9 C18:1). Concentrations and yields of individual FA are reported in Supplemental Tables S3 and S4, respectively (<https://doi.org/10.3168/jds.2020-19311>).

²Control (CON) = diet not supplemented with FA; 80:10 = 1.5% of FA supplement blend to provide ~80% C16:0 and 10% *cis*-9 C18:1; 70:20 = 1.5% of FA supplement blend to provide ~70% C16:0 and 20% *cis*-9 C18:1; 60:30 = 1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1.

³*P*-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets: (80:10 + 70:20 + 60:30)/3]; linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

in commercially available FA supplements fed to dairy cows, and these FA normally comprise the majority of FA present in milk fat and adipose tissue (Jensen, 2002, Douglas et al., 2007). Our recent research has indicated that altering the dietary ratio of C16:0 and *cis*-9 C18:1 may alter nutrient partitioning between the mammary gland and adipose tissue in postpeak cows (de Souza et al., 2018; de Souza et al., 2019b). Because metabolic state plays a critical role in energy partitioning, the aim of our current study was to evaluate the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in supplemental fat on production responses of early-lactation dairy cows, whereas our companion paper focuses on nutrient digestibility, energy balance, and metabolism of early-lactation cows (de Souza et al., 2021).

Some authors suggest that feeding FA to cows immediately postpartum may depress feed intake (Kuhla et al., 2016), because DMI is likely primarily controlled by mechanisms related to oxidation of fuels in the liver in the early postpartum period (Allen and Piantoni, 2013). In our study, we did not observe differences in feed intake between the CON and 80:10 treatment. Similarly, de Souza and Lock (2019) fed a C16:0 supplement and reported no differences compared with a nonfat supplemented control diet for DMI when the supplement was fed in the immediate postpartum period (up to 24 DIM) or early-lactation postpartum period (up to 67 DIM). Importantly, the effect of FA on feed intake is associated with the FA profile of the supplement fed (Allen, 2000; Rabiee et al., 2012) with DMI decreasing linearly as the degree of unsaturation of supplemental fat increased (Drackley et al., 1992; Harvatine and Allen, 2006). However, in our study we unexpectedly observed that DMI increased as we increased *cis*-9 C18:1 in the FA

treatments. Also, previous studies reported that feeding a saturated prilled FA supplement (C16:0 + C18:0) increased DMI in cows in the immediate postpartum and early lactation (Moallem et al., 2007; Piantoni et al., 2015a). Interestingly, we also observed that increasing *cis*-9 C18:1 in the FA treatments increased plasma insulin and decreased NEFA in the immediate postpartum (de Souza et al., 2021). Piantoni et al. (2015b) reported that greater reductions in plasma NEFA concentrations after feeding were positively related to greater intakes in early postpartum cows, suggesting that decreased β -oxidation in the liver might allow for higher DMI. Plasma insulin concentration increased during and after meals, resulting in decreased lipolysis and plasma NEFA concentrations (Allen et al., 2005). Therefore, the increase in DMI observed in our study as we increased *cis*-9 C18:1 in the FA treatments may be related to a decreased flux of fuels to the liver that could have potentially decreased satiety and increased DMI (Allen et al., 2009).

In our study, FA supplementation increased milk yield in the immediate postpartum, but no differences were observed among the FA treatments. Milk yield responses to FA supplementation in the immediate postpartum have been inconsistent which is most likely associated with the FA profile of supplemental fat (e.g., de Souza and Lock, 2019; Piantoni et al., 2015a; Greco et al., 2015). de Souza and Lock (2019) fed a C16:0 supplement and reported no differences compared with a nonfat supplemented control diet for milk yield when the supplement was fed up to 24 d postpartum, whereas milk yield increased by 3.45 kg/d when the supplement was fed from 25 to 67 d postpartum. Piantoni et al. (2015a) observed that feeding a saturated prilled FA

supplement (C16:0 + C18:0) tended to decrease milk yield by 3.1 kg/d in cows in the immediate postpartum period (1–29 DIM). In contrast, Moallem et al. (2000) reported that feeding Ca salts of palm FA supplement increased by milk yield 2.2 kg/d without affecting DMI during the first 150 d of lactation. However, the effects on milk yield and DMI were reported as least squares means for the whole 150 d in lactation so that the effect of FA supplementation during the immediate postpartum on production performance cannot be separated.

We observed that, compared with CON, the FA-supplemented treatments increased milk fat yield, 3.5% FCM, and ECM, and the treatment differences in these variables was consistent across time. In agreement with this, previous studies have observed that C16:0 supplementation increased 3.5% FCM and ECM during early lactation (de Souza and Lock, 2019) and in postpeak cows (de Souza and Lock, 2018; Western et al., 2020). Additionally, de Souza et al. (2018) observed that, compared with a control diet, ECM and milk energy output increased in postpeak cows when fed a FA blend containing primarily C16:0 (80% C16:0), but not with a FA blend containing a combination of C16:0 and *cis*-9 C18:1 (45% C16:0 and 35% *cis*-9 C18:1). In contrast, Piantoni et al. (2015a) observed that feeding a saturated prilled FA supplement (C16:0 + C18:0) did not affect the yield of 3.5% FCM and ECM in cows in the immediate postpartum period (1 to 29 DIM). Similarly, Moallem et al. (2007) fed a saturated prilled FA (C16:0 + C18:0) and showed that the supplement did not affect 3.5% FCM or milk energy output. Feeding a Ca salts of palm FA supplement (2.6% diet DM) from parturition to 120 DIM increased 3.5% FCM in dairy cows, but also increased BW loss (Sklan et al., 1991, 1994). Therefore, changes in the yield of milk components are directly related to the FA profile of supplemental fed in these diets.

In our study, we observed that the increase in milk fat yield due to FA supplementation was mostly explained by changes in the yield of 16-carbon FA in milk fat. Also, increasing *cis*-9 C18:1 in FA treatments quadratically affected mixed FA and C16:0 yield because their yield was highest with the 80:10 treatment. These findings agree with previous studies feeding supplements with C16:0 compared with a control diet in postpeak (Lock et al., 2013; Rico et al., 2017) and early-lactation cows (de Souza and Lock, 2019). Hansen and Knudsen (1987) reported that C16:0 stimulated de novo FA synthesis and incorporation into triglycerides in dispersed goat mammary epithelial cells, whereas other FA (C18:0, C18:1, and C18:2) had no effect. Also, a higher preference (8- to 10-fold) was shown for C16:0 as a substrate for glycerol-3-phosphate acyltransferase (**GPAT**), which esterifies FA at sn-1 position to start

TAG synthesis, than for C18:0 or *cis*-9 C18:1 (Kinsella and Gross, 1973). In a meta-analysis, Dorea and Armentano (2017) observed a negative relationship between dietary *cis*-9 C18:1 content and de novo milk FA yield. This 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. In our study, increasing *cis*-9 C18:1 in FA treatments linearly increased the yield of C18:0 and *cis*-9 C18:1 without affecting de novo yield. de Souza et al. (2019b) suggested an interdependence between de novo and preformed FA when high-producing cows received increasing levels of *cis*-9 C18:1 in the diet increasing milk fat yield, whereas a substitution effect occurred in low producing cows.

We observed that our 80:10 treatment increased the yield of milk and ECM, but also increased BW and BCS losses in the immediate postpartum. Similarly, we previously reported greater BW loss and lower plasma insulin levels for cows fed a C16:0 supplement during the first 24 d postpartum (de Souza and Lock, 2019; de Souza et al., 2019a). Interestingly, we observed that increasing *cis*-9 C18:1 in FA treatments increased the yield of milk and ECM without changes in body reserve mobilization when compared with CON. This difference in nutrient partitioning is likely driven by insulin, as we observed that increasing *cis*-9 C18:1 in FA treatments increased plasma insulin concentration (de Souza et al., 2021). Although, to our knowledge, this has not been studied in cows, previous studies using rats observed that *cis*-9 C18:1 stimulated insulin secretion from pancreatic β -cells (Itoh et al., 2003; Fujiwara et al., 2005). Elevated insulin concentrations would then reduce plasma NEFA through inhibiting lipolysis or increasing lipogenesis (Vernon, 2005). Additionally, we observed in our study that increasing *cis*-9 C18:1 in FA treatments linearly increased the concentration and yield of some *trans* FA (*trans* 6–8 and *trans*-9 C18:1). Boerman et al. (2015) observed a positive correlation between milk fat *trans*-10 C18:1 content and change in BCS, however milk *trans*-10 C18:1 content was not associated with milk fat yield. This is likely associated with repartitioning of energy by reducing milk energy output and increasing body fat reserves. Harvatine et al. (2009) reported that during abomasal infusions of *trans*-10, *cis*-12 C18:2, there was a downregulation of lipogenic enzymes in mammary tissue, and an increase in the expression of lipogenic enzymes in adipose tissue. In our study we did not detect levels of *trans*-10, *cis*-12 C18:2 in milk fat for the majority of our samples, but it is important to consider that other FA produced as intermediates during rumen biohydrogenation have

been shown to reduce milk fat (Bauman et al., 2011) and potentially may be involved with energy partitioning. Additionally, it should be noted that although we increased dietary *cis*-9 C18:1, it is likely that this treatment increased rumen outflow of other 18-carbon FA so that it is unclear if these results are associated with an overall effect of 18-carbon FA or a specific FA. Further research is needed to determine whether a higher amount of 18-carbon FA or a higher amount of a specific FA is related to energy partitioning toward body reserves, and to determine the mechanisms associated with it.

It has been demonstrated that changes in production responses during a treatment period can influence subsequent lactation performance (Jorgensen et al., 2016). One of our objectives was to evaluate the potential carryover effects of FA supplementation during the immediate postpartum on production responses throughout early lactation. Interestingly, in our study the fat-supplemented diets fed during the immediate postpartum period had a positive carryover effect during early lactation while fed a common diet. The yield of milk and milk components, 3.5% FCM, and ECM were higher during the carryover period for cows that received FA-supplemented diets compared with CON during early postpartum. Piantoni et al. (2015a) observed that feeding a saturated prilled FA supplement (C16:0 + C18:0) did not affect the yield of 3.5% FCM, and ECM in the immediately postpartum (1 to 29 DIM), but FA supplementation had a pronounced carryover effect (30 to 67 DIM) decreasing both 3.5% FCM and ECM in a low forage diet. With grazing cows, supplementing a Ca-salts of palm FA supplement from 3 to 16 wks of lactation increased cumulative milk yield throughout lactation by 8 to 12% (Batistel et al., 2017; de Souza et al., 2017). Possible explanations for the carryover effect on milk yield involve either an increase in mammary cell number (Akers, 2002) or cell secretory activity (Nørgaard et al., 2005). Also, the development of epithelial cell populations in the mammary gland is primarily regulated by ovarian steroids including estrogen (Arendt and Kuperwasser, 2015). Flaxseed oil was shown to alter mammary development, modify mammary gland morphology and increase the number of estradiol receptor binding sites in the mammary gland of mice (Hilakivi-Clarke et al., 1998). Feeding prepubertal heifers with soybean oil slightly improved mammary development but did not affect the yields of milk and milk components during their first lactation (Thibault et al., 2003). Thus, although FA supplementation has the potential to affect future milk production, further studies are needed to understand factors associated with carryover effects, and to determine the

duration and magnitude of this under different dietary conditions.

CONCLUSIONS

Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased milk yield and ECM compared with a control diet not supplemented with FA. Increasing *cis*-9 C18:1 in the FA supplement increased DMI and reduced BW and BCS losses. Additionally, the fat-supplemented diets fed during the immediate postpartum period had a positive carryover effect during early lactation, when cows were fed a common diet. The yield of milk and milk components, 3.5% FCM, and ECM were higher during the carryover period for cows that received FA-supplemented diets compared with CON during the early postpartum period.

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