



## Altering the ratio of dietary palmitic and oleic acids affects nutrient digestibility, metabolism, and energy balance during the immediate postpartum in dairy cows

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### ABSTRACT

This article is the second from an experiment that determined the effects of altering the dietary ratio of palmitic (C16:0) and oleic (*cis*-9 C18:1) acids on digestibility, production, and metabolic responses of dairy cows during the immediate postpartum. This article elaborates on the effect of these diets on nutrient digestibility, energy balance, and metabolism. Fifty-six multiparous cows were used in a randomized complete block design and randomly assigned to 1 of 4 treatments fed from 1 to 24 d in milk. 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 dry matter by replacing soyhulls in the CON diet. Three preplanned contrasts were used to compare treatment differences: (1) CON versus FA-supplemented diets, (80:10 + 70:20 + 60:30)/3; (2) the linear effect of *cis*-9 C18:1 inclusion in diets; and (3) the quadratic effect of *cis*-9 C18:1 inclusion in diets. The FA-supplemented diets increased digestibility of dry matter, neutral detergent fiber, 18-carbon FA, and total FA compared with CON. We observed a tendency for an interaction between treatment and time for the digestibility of 18-carbon and total FA because the difference in digestibility between CON and 60:30 treatments tended to increase over time. Increasing dietary *cis*-9 C18:1 increased linearly the digestibility of dry matter, neutral detergent fiber, 16-carbon, 18-carbon, and total FA. Interestingly, total absorbed FA was posi-

tively related to milk, milk fat yield, energy-corrected milk, plasma insulin, and albumin, and negatively related to plasma nonesterified FA (NEFA) and body weight loss. The FA-supplemented diets increased intake of digestible energy, metabolizable energy, and net energy for lactation compared with CON. Compared with CON, FA-supplemented diets increased milk energy output and tended to increase negative energy balance. Increasing dietary *cis*-9 C18:1 increased intake of digestible energy, metabolizable energy, and net energy for lactation. Although increasing dietary *cis*-9 C18:1 did not affect milk energy output and energy for maintenance, increasing dietary *cis*-9 C18:1 improved energy balance. Compared with CON, FA-supplemented diets increased plasma insulin, but we did not observe differences between CON and FA-supplemented diets for NEFA and albumin. Increasing *cis*-9 C18:1 in FA treatments linearly decreased plasma NEFA and tended to linearly increase insulin and  $\beta$ -hydroxybutyrate. During the carryover period, no treatment differences in blood metabolites were observed. Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased nutrient digestibility, energy intake, and milk energy output compared with a non-fat-supplemented control diet. Increasing dietary *cis*-9 C18:1 increased energy intake, reduced markers of body fat mobilization, and improved energy balance during the immediate postpartum.

**Key words:** palmitic acid, oleic acid, energy balance, nutrient digestibility

### INTRODUCTION

Lactogenesis, uterine involution, and pronounced changes in endocrine function and energy balance create a unique set of challenges that trigger major adaptive changes in the metabolic function of dairy cows during early lactation (Bradford et al., 2015). Therefore, dairy cows undergo large metabolic adaptations in glucose, AA, fatty acid (FA), and mineral metabolism to support lactation and avoid metabolic dysfunction imme-

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diately postpartum (Overton et al., 2017). The major nutritional challenge in early lactation is to meet the increasing requirements for energy and key nutrients while feed intake is limited. As part of the metabolic adaptive mechanisms, body fat reserves are mobilized around parturition, due to reduced plasma insulin concentration (Zachut et al., 2013), low insulin sensitivity of extra-hepatic tissues (Bell, 1995), and increased catecholamine-stimulated lipolysis (Contreras et al., 2017). Increased mobilization of body reserves results in increased plasma nonesterified fatty acid (NEFA) concentrations (González et al., 2011), which can alter immune function and increase the risk and severity of both metabolic and infectious diseases (Sordillo et al., 2009; Sordillo, 2016). Furthermore, the control of feed intake is likely dominated by hepatic oxidation of NEFA during the immediate postpartum period (Allen et al., 2009), and therefore, the increased NEFA supply to the liver can further decrease energy intake, thereby intensifying negative energy balance (Allen and Piantoni, 2013). Thus, considering that nutrient status plays a critical role during early lactation, nutrition-based management strategies to increase energy intake and minimize negative energy balance are important to the health and productivity of dairy cattle.

Supplemental fat can be used as a strategy to increase energy intake, but limited research is available, and results are inconsistent regarding the effects of FA supplementation on energy balance and metabolism in early-lactation cows. Piantoni et al. (2015b) observed that feeding a saturated FA supplement [37% palmitic acid (C16:0) + 46% stearic acid (C18:0)] at 2.0% of diet DM during the first 4 wk postpartum interacted with forage NDF level on the response of dairy cows; when the FA supplement was fed in a low forage diet (20% of forage NDF), it increased energy intake and energy balance and reduced body fat mobilization at the expense of milk yield. The high forage diet with supplemental FA increased DMI and tended to decrease BCS loss compared with the same diet without FA supplementation. In a recent study with early-lactation cows, feeding a C16:0-enriched supplement at 1.5% of diet DM during the first 24 d postpartum increased the yield of milk fat, ECM, and BW loss; did not affect DMI; and resulted in a more negative energy balance in dairy cows (de Souza and Lock, 2019; de Souza et al., 2019a). Therefore, the FA profile of supplemental fat is a major factor affecting production responses and metabolism of early-lactation cows.

In postpeak cows, we recently observed that feeding an FA blend with a high content of C16:0 (80% C16:0) increased milk energy output and energy partitioning toward milk, whereas feeding a FA blend containing

45% C16:0 and 35% oleic acid (*cis*-9 C18:1) increased energy allocated to BW and the partitioning of energy to BW compared with a control diet not supplemented with FA (de Souza et al., 2018). Interestingly, feeding *cis*-9 C18:1 not only increased BW gain in postpeak cows but also increased plasma insulin compared with control diets and other FA supplements (de Souza et al., 2018, 2019b). Lipolysis and lipogenesis are influenced by circulating insulin concentrations, as well as by the responsiveness and sensitivity of adipose tissue to insulin (De Koster and Opsomer, 2013). Thus, increases in plasma insulin or insulin sensitivity during early lactation may lead to decreased body fat mobilization and improvements in energy balance. However, to our knowledge, the effect of feeding *cis*-9 C18:1 alone or in combination with other FA has not been evaluated in early lactation cows.

Therefore, we hypothesized that increasing the amount of *cis*-9 C18:1 in supplemental fat would reduce milk energy output due to differences in milk fat yield responses, and that feeding *cis*-9 C18:1 would reduce body reserve mobilization and improve energy balance in early lactation. Our objective was to determine the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 on nutrient digestibility, metabolism, and energy balance of early lactation dairy cows during the immediate postpartum period.

## MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). This article is the second from an experiment that evaluated 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. This article focuses on the effect of these diets on nutrient digestibility, energy intake and balance, and plasma metabolites and hormones. The companion paper (de Souza et al., 2021) describes treatment effects on DMI, yield of milk and milk components, BW and BCS, and milk FA profile.

### 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 (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), and parity (up to 1 lactation difference). The BCS used to block cows was

the last measurement before parturition. 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 supplement blends (Nutracor and Nutracal, Wawasan Agrolipids, Johor, Malaysia). 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, replacing soyhulls in the CON diet. Treatment diets were mixed daily in a tumble-mixer and were fed from the morning following parturition. From d 25 to 63 postpartum (carryover period), all cows were offered a common diet, mixed daily in a mixer wagon. Treatment diets contained 23% forage NDF, 24.6% starch, and 16.7% CP. Carryover diet contained 20.3% forage NDF, 27.6% starch, and 16.9% CP. The ingredient and nutrient composition of the diets fed as TMR, including the close-up ration for reference, as well as a summary of all health incidents during the treatment period are reported in the companion article (de Souza et al., 2021).

### Data and Sample Collection

Milk yield, amount of feed offered and refused, BW, and BCS were recorded, and samples of milk, feed ingredients, and orts were collected and stored as described in de Souza et al. (2021). Samples and body measurements were collected or recorded on the same day of the week during the entire experiment, so that all collection days are  $\pm 3$  d.

On d 5, 12, and 19 postpartum, fecal samples (500 g) were collected every 6 h, representing every 6 h of a 24-h period to account for diurnal variation, for nutrient digestibility analysis. Feces were stored in sealed plastic cups at  $-20^{\circ}\text{C}$  until dried, ground, and composited per cow per sampling day. Blood samples were collected on d 5, 12, 19, and 33 postpartum by venipuncture of coccygeal vessels within 1 h before feeding. Blood was collected into 2 evacuated tubes, 1 containing potassium EDTA, and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor. Both were centrifuged at  $2,000 \times g$  for 15 min at  $4^{\circ}\text{C}$  immediately after sample collection, and plasma was harvested and stored at  $-20^{\circ}\text{C}$  until analysis.

### Sample Analysis

Diet ingredients, orts, and fecal samples were dried at  $55^{\circ}\text{C}$  in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Feed ingredients and orts were analyzed for NDF, CP, starch, and FA concentrations as described by Boerman et al. (2017). Fecal samples were analyzed for NDF and FA concentrations as described by Boerman et al. (2017). Gross energy (**GE**) was assayed by bomb calorimeter (Parr Instrument Inc., Moline, IL) in all samples. Indigestible NDF was used as an internal marker to estimate fecal output to determine the apparent total-tract digestibility of nutrients (Cochran et al., 1986). Indigestible NDF was estimated as NDF after a 240-h in vitro fermentation (Goering and Van Soest, 1970). Serum concentrations of NEFA, BHB, and albumin were determined using an Olympus AU640e chemistry analyzer (Olympus America, Center Valley, PA) at the Diagnostic Center for Population and Animal Health of Michigan State University (Lansing, MI) with intra- and interassay CV  $<3\%$ . Serum insulin concentrations were determined by ELISA (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden) with intra- and inter-assay CV  $<3\%$ .

Energy intake and balance were calculated using equations (NRC, 2001) according to Harvatine and Allen (2006). Digestible energy (**DE**) intake = GE intake  $\times$  GE digestibility. The  $\text{NE}_L$  intake was calculated from DE according to NRC (2001) as  $\text{NE}_L(\text{intake}) = 0.703 \times \text{ME}(\text{intake}) - 0.19 + \{[(0.097 \times \text{ME}(\text{intake}) + 0.19)/97] \times [\text{ether extract} - 3]\}$ ;  $\text{ME}(\text{intake}) = 1.01 \times (\text{DE}(\text{intake})) - 0.45 + 0.0046 \times (\text{ether extract} - 3)$  (NRC, 2001). Milk energy output (Mcal/d) was calculated according to NRC (2001) as milk energy output (Mcal/d) =  $[9.29 \times \text{fat}(\text{kg}) + 5.63 \times \text{true protein}(\text{kg}) + 3.95 \times \text{lactose}(\text{kg})]$ , where each component was based on the average output of a cow during the week. The  $\text{NE}_m$  (Mcal/d) was calculated as  $0.08 \text{ Mcal/kg} \times \text{BW}(\text{kg})^{0.75}$  and energy balance (Mcal/d) determined as  $\text{NE}_L$  intake (Mcal/d)  $-$  milk  $\text{NE}_L$  (Mcal/d)  $-$   $\text{NE}_L$  maintenance requirement (Mcal/d) (NRC, 2001). The efficiency of energy utilization for milk was calculated as milk energy output/DE intake ( $\text{NE}_L$  milk/DEI). The efficiency of energy utilization for production was calculated as milk (energy output + energy for maintenance)/DE intake ( $\text{NE}_L$  production/DEI).

### Statistical Analysis

Data were analyzed 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 the week being the repeated measurement.

The model used for the treatment period (1–24 DIM) 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,  $\mu$  = 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 during the treatment by time interaction, and  $e_{ijkl}$  = residual error.

Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest BIC for most of the variables measured. A reduced model was used for analysis of blood metabolites variables collected in the carryover period without the effect of time in the model because a single time point was collected. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals vs. 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: (1) CON versus

FA-supplemented diets, (80:10 + 70:20 + 60:30)/3; (2) the linear effect of *cis*-9 C18:1 inclusion in diet; and (3) the quadratic effect of *cis*-9 C18:1 inclusion in diet.

## RESULTS

### Nutrient Digestibility

The FA-supplemented diets did not affect DMI ( $P = 0.14$ ; Table 1) but tended to increase NDF intake ( $P = 0.06$ ) compared with CON. Also, compared with CON, FA-supplemented diets increased intake of 16-carbon ( $P < 0.01$ ), 18-carbon ( $P < 0.01$ ), and total FA ( $P < 0.01$ ). The FA-supplemented diets increased digestibility of DM ( $P = 0.02$ ), NDF ( $P < 0.01$ ), 18-carbon ( $P < 0.01$ ), and total FA ( $P < 0.01$ ) compared with CON. Additionally, compared with CON, FA-supplemented diets increased absorbed 16-carbon ( $P < 0.01$ ), 18-carbon ( $P < 0.01$ ), and total FA ( $P < 0.01$ ).

Increasing dietary *cis*-9 C18:1 increased 16-carbon (quadratic,  $P < 0.01$ ), 18-carbon (linear,  $P < 0.01$ ), and total FA intakes (quadratic,  $P < 0.01$ ). Additionally, increasing dietary *cis*-9 C18:1 increased digestibility of DM (linear,  $P < 0.01$ ), NDF (linear,  $P < 0.01$ ), 16-carbon (linear,  $P = 0.04$ ), 18-carbon (linear,  $P < 0.01$ ), and total FA (linear,  $P < 0.01$ ). Increasing dietary *cis*-9 C18:1 increased absorbed 16-carbon (quadratic,  $P < 0.01$ ), 18-carbon (linear,  $P < 0.01$ ), and total FA (quadratic,  $P < 0.01$ ).

**Table 1.** Nutrient intake and total-tract nutrient digestibility for cows fed treatment diets during the treatment period (d 1 to 24 postpartum)

Item	Treatment <sup>1</sup>				SEM	Contrast <sup>2</sup>			P-value <sup>3</sup>	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt × Time
Intake, kg										
DM	20.3	20.7	20.9	21.8	0.48	0.14	0.03	0.51	<0.01	0.97
NDF	4.74	5.00	5.17	5.32	0.25	0.06	0.15	0.34	<0.01	0.53
Intake, g/d										
Total FA	316	669	643	688	31.7	<0.01	<0.01	<0.01	<0.01	0.36
16-carbon	54	311	266	256	13.7	<0.01	<0.01	<0.01	<0.01	0.30
18-carbon	253	338	360	417	17.7	<0.01	<0.01	0.42	<0.01	0.30
Digestibility, %										
DM	69.2	69.9	70.2	71.0	0.52	0.02	<0.01	0.98	0.42	0.14
NDF	37.6	39.9	39.9	41.3	0.76	<0.01	<0.01	0.53	0.43	0.17
Total FA	82.3	82.5	83.8	84.9	0.97	<0.01	<0.01	0.12	0.46	0.15
16-carbon	78.2	77.7	79.6	81.1	1.18	0.36	0.04	0.37	0.60	0.29
18-carbon	85.7	88.0	88.5	88.7	0.92	<0.01	<0.01	0.98	0.25	0.10
Absorbed FA, g/d										
Total FA	268	554	538	585	26.5	<0.01	<0.01	<0.01	<0.01	0.44
16-carbon	43	246	211	208	10.9	<0.01	<0.01	<0.01	<0.01	0.34
18-carbon	223	297	319	370	15.7	<0.01	<0.01	0.47	<0.01	0.45

<sup>1</sup>Treatments were: CON (control; no supplemental fat); 80:10 [1.5% of fatty acid (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); and 60:30 (1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1).

<sup>2</sup>P-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets: (80:10 + 70:20 + 60:30)/3]; and linear and quadratic effects of *cis*-9 C18:1 inclusion in supplemental fat.

<sup>3</sup>Trt = treatment.

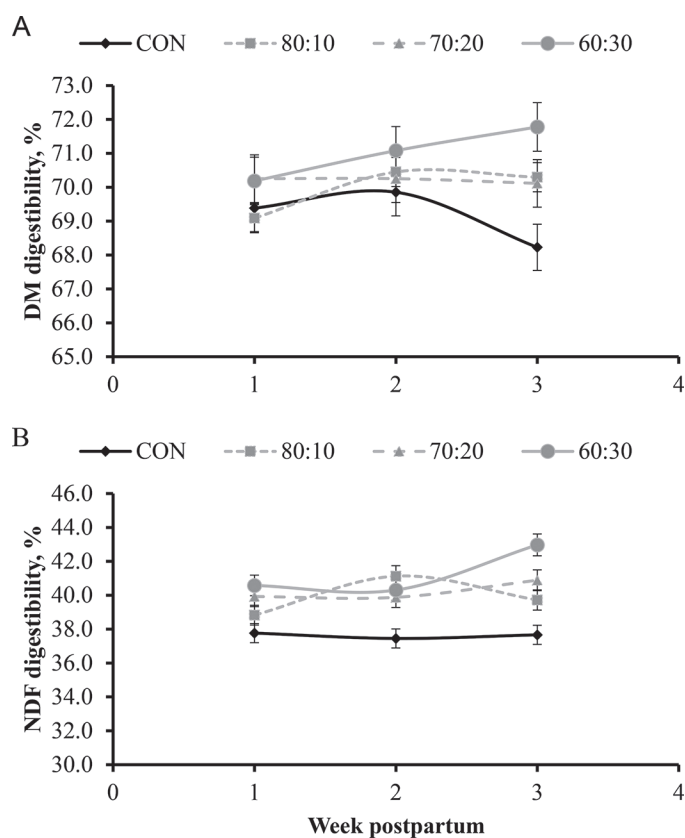


Overall, nutrient digestibility was not affected by time ( $P > 0.25$ ). We observed a tendency for an interaction between treatment and time for the digestibility of DM ( $P = 0.14$ ) because the difference in digestibility between CON and 60:30 tended to increase over time (Figure 1). We also observed a tendency for an interaction between treatment and time for the digestibility of 18-carbon ( $P = 0.10$ ) and total FA ( $P = 0.15$ ), because the difference in digestibility between CON and 60:30 tended to increase over time (Figure 2).

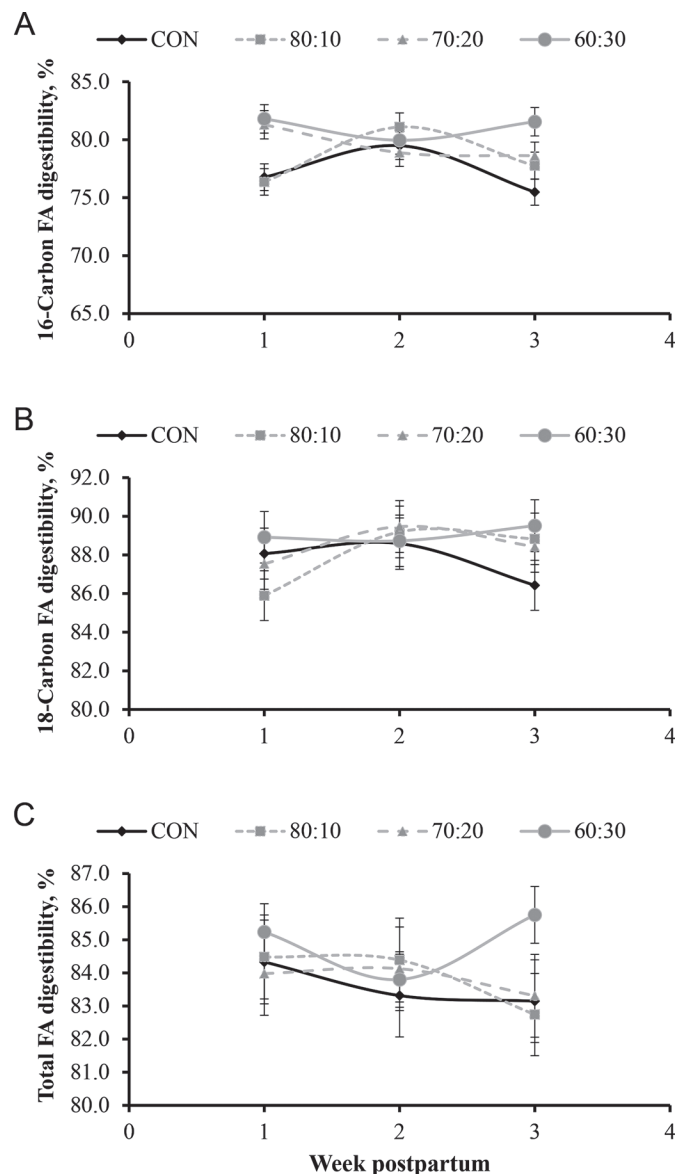
### Energy Intake and Energy Balance

The FA-supplemented diets increased intake of DE ( $P = 0.04$ ; Table 2), ME ( $P = 0.04$ ), and  $NE_L$  ( $P = 0.05$ ) compared with CON. Compared with CON, FA-

supplemented diets increased milk energy output ( $P = 0.04$ ) and tended to make energy balance more negative ( $P = 0.08$ ). We did not observe treatment differences for the efficiency of energy utilization for milk ( $P =$



**Figure 1.** Effects of dietary treatments on digestibility of DM (A) and NDF (B) over time during the treatment (1–24 DIM). Diets fed during the treatment period were CON (control diet not supplemented with FA); 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 60:30 (60% C16:0 + 30% *cis*-9 C18:1). Compared with CON, FA-supplemented diets increased digestibility of DM ( $P = 0.02$ ) and NDF ( $P < 0.01$ ). We observed a tendency for an interaction between treatment and time for the digestibility of DM ( $P = 0.14$ ) because the difference in digestibility between CON and 60:30 tended to increase over time. Additionally, increasing dietary *cis*-9 C18:1 increased digestibility of DM (linear,  $P < 0.01$ ) and NDF (linear,  $P < 0.01$ ). Error bars indicate SEM.



**Figure 2.** Effects of dietary treatments on digestibility of 16-carbon (A), 18-carbon (B), and total FA (C) over time during the treatment (1–24 DIM). Diets fed during the treatment period were CON (control diet not supplemented with FA); 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The FA-supplemented diets increased digestibility of 18-carbon ( $P < 0.01$ ) and total FA ( $P < 0.01$ ) compared with CON. We observed a tendency for an interaction between treatment and time for the digestibility of 18-carbon ( $P = 0.10$ ) and total FA ( $P = 0.15$ ) because the difference in digestibility between CON and 60:30 tended to increase over time. Additionally, increasing dietary *cis*-9 C18:1 increased digestibility of 16-carbon (linear,  $P = 0.04$ ), 18-carbon (linear,  $P < 0.01$ ), and total FA (linear,  $P < 0.01$ ). Error bars indicate SEM.

**Table 2.** Energy intake, energy output and balance, and efficiency of energy utilization for cows fed treatment diets during the treatment period (d 1 to 24 postpartum)

Item	Treatment <sup>1</sup>				SEM	Contrast <sup>2</sup>			P-value <sup>3</sup>	
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic	Time	Trt × Time
Energy intake, Mcal/d										
DE <sup>4</sup>	59.4	62.3	62.5	64.4	1.51	0.04	0.02	0.73	<0.01	0.91
ME <sup>5</sup>	51.7	53.5	54.1	55.5	1.11	0.04	0.02	0.86	<0.01	0.87
NE <sub>L</sub> <sup>6</sup>	31.9	33.7	34.2	35.0	0.63	0.05	0.01	0.99	<0.01	0.75
Energy output, Mcal/d										
Milk <sup>7</sup>	33.9	37.3	36.4	36.6	1.20	0.04	0.17	0.17	<0.01	0.78
Maintenance <sup>8</sup>	10.9	10.7	10.9	11.1	0.20	0.99	0.32	0.29	<0.01	0.05
Energy balance, <sup>9</sup> Mcal/d	-11.7	-14.9	-13.2	-12.7	0.95	0.08	0.77	0.04	<0.01	0.22
Efficiency										
NE <sub>L</sub> milk/DE intake	0.566	0.596	0.586	0.568	0.02	0.33	0.92	0.09	0.28	0.90
NE <sub>L</sub> production/DE intake <sup>10</sup>	0.750	0.768	0.761	0.741	0.02	0.74	0.63	0.23	0.02	0.86

<sup>1</sup>Treatments were: CON (control; no supplemental fat); 80:10 [1.5% of fatty acid (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); and 60:30 (1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1).

<sup>2</sup>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.

<sup>3</sup>Trt = treatment.

<sup>4</sup>Digestible energy (DE) intake = gross energy intake (Mcal/d) × gross energy digestibility.

<sup>5</sup>Metabolizable energy intake = 1.01 × (DE intake - 0.45) + 0.0046 × (ether extract - 3); NRC (2001).

<sup>6</sup>Net energy of lactation intake: NE<sub>L</sub> intake = 0.703 × ME intake - 0.19 + {[(0.097 × ME intake + 0.19)/97] × [ether extract - 3]}.

<sup>7</sup>Milk NE<sub>L</sub> (Mcal/d) = milk yield (kg/d) × [(fat % × 0.0929) + (true protein % × 0.0563) + (lactose % × 0.0395)]; NRC (2001).

<sup>8</sup>NE<sub>L</sub> maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg)<sup>0.75</sup>; NRC (2001).

<sup>9</sup>Energy balance (Mcal/d) = NE<sub>L</sub> intake (Mcal/d) - milk NE<sub>L</sub> (Mcal/d) - NE<sub>L</sub> maintenance requirement (Mcal/d).

<sup>10</sup>NE<sub>L</sub> production = milk NE<sub>L</sub> + NE<sub>L</sub> required for maintenance.

0.33) and production ( $P = 0.74$ ). Overall, DE intake and milk energy output increased over time ( $P < 0.01$ ), and the differences across treatments were consistent over time for these variables (Figure 3).

Increasing dietary *cis*-9 C18:1 increased intake of DE (linear,  $P = 0.02$ ), ME (linear,  $P = 0.02$ ), and NE<sub>L</sub> (linear  $P = 0.01$ ). Although increasing dietary *cis*-9 C18:1 did not affect milk energy output ( $P > 0.17$ ) or energy for maintenance ( $P > 0.29$ ), increasing dietary *cis*-9 C18:1 improved energy balance (quadratic,  $P = 0.04$ ). Increasing dietary *cis*-9 C18:1 tended to increase the efficiency of energy utilization for milk (quadratic,  $P = 0.09$ ) but did not affect energy utilization for production ( $P > 0.23$ ).

### Plasma Insulin and Metabolites During the Treatment Period

Compared with CON, FA-supplemented diets increased plasma insulin ( $P = 0.02$ ; Table 3). We did not observe differences between CON and FA-supplemented diets for plasma NEFA ( $P = 0.57$ ), BHB ( $P = 0.14$ ), or albumin ( $P = 0.11$ ). Increasing *cis*-9 C18:1 in FA treatments linearly decreased plasma NEFA ( $P =$

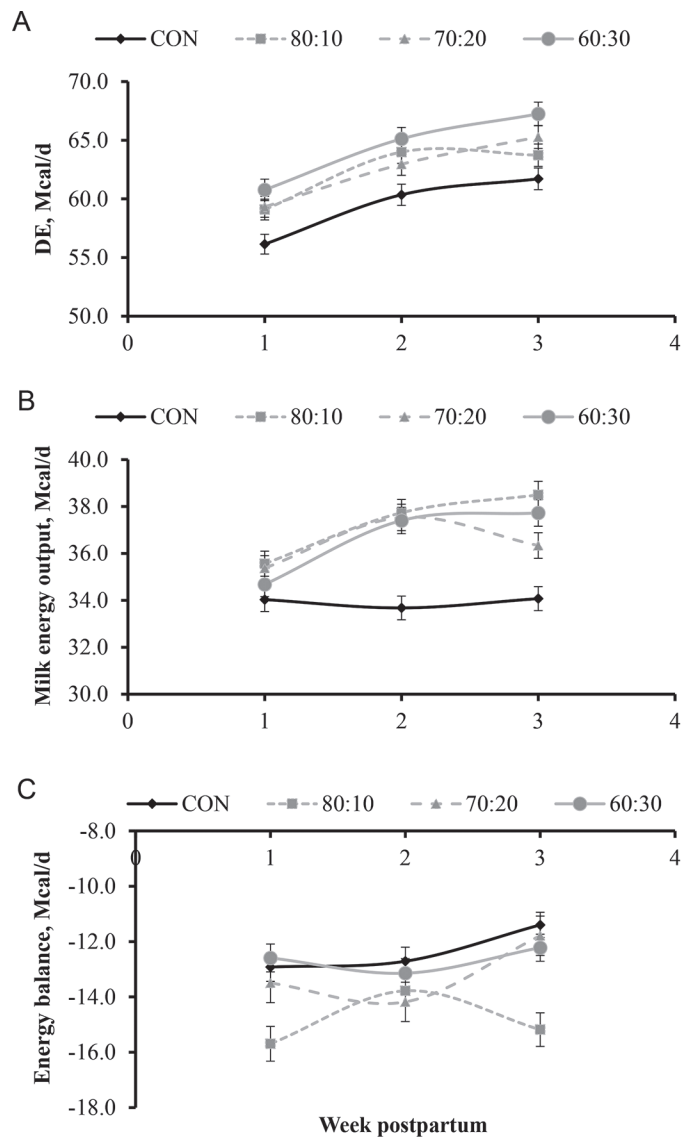
0.03; Table 3), tended to linearly increase insulin ( $P = 0.07$ ) and BHB ( $P = 0.10$ ), but did not affect albumin ( $P > 0.10$ ). Also, we tended to observe an interaction between treatment and time for BHB ( $P = 0.15$ ) due to FA-supplemented diets increasing BHB compared with CON at wk 3 (Figure 4).

### Plasma Insulin and Metabolites During the Carryover Period

During the carryover period, blood metabolites were only evaluated at 1 time point (d 33). We did not observe difference between CON and FA-supplemented diets or among the FA treatments for the metabolites evaluated ( $P > 0.10$ ; Table 4).

### Correlations

We used Pearson correlation coefficients to assess relationships between production and metabolic variables (Table 5). Interestingly, total absorbed FA was positively related to milk yield ( $P < 0.01$ ;  $r = 0.42$ ), milk fat yield ( $P < 0.01$ ;  $r = 0.35$ ), ECM yield ( $P < 0.01$ ;  $r = 0.37$ ), plasma insulin ( $P < 0.01$ ;  $r = 0.28$ ), and



**Figure 3.** Effects of dietary treatments on digestible energy (DE) intake (A), milk energy output (B), and energy balance (C) over time during the treatment (1–24 DIM). Diets fed during the treatment period were CON (control diet not supplemented with FA); 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 60:30 (60% C16:0 + 30% *cis*-9 C18:1). The FA-supplemented diets increased intake of DE ( $P = 0.04$ ) and milk energy output ( $P = 0.04$ ), and tended to reduce energy balance ( $P = 0.08$ ) compared with CON. Overall, DE intake, milk energy output, and energy balance increased over time ( $P < 0.01$ ), and the differences across treatments were consistent over time for these variables. Increasing dietary *cis*-9 C18:1 increased intake of DE (linear,  $P = 0.02$ ) and improved energy balance (quadratic,  $P = 0.04$ ). Error bars indicate SEM.

plasma albumin ( $P < 0.01$ ;  $r = 0.29$ ), and negatively related to plasma NEFA ( $P = 0.03$ ;  $r = -0.18$ ) and BW loss ( $P = 0.08$ ;  $r = -0.14$ ). We observed that DE intake was positively related to DMI ( $P < 0.01$ ;  $r = 0.79$ ), milk yield ( $P < 0.01$ ;  $r = 0.60$ ), milk fat yield ( $P < 0.01$ ;  $r = 0.29$ ), ECM ( $P < 0.01$ ;  $r = 0.29$ ), plasma

insulin ( $P = 0.01$ ;  $r = 0.22$ ), and plasma albumin ( $P < 0.01$ ;  $r = 0.36$ ), and negatively related to plasma NEFA ( $P < 0.01$ ;  $r = -0.25$ ) and BHB ( $P = 0.02$ ;  $r = -0.19$ ). Milk energy output was positively related to total absorbed FA ( $P < 0.01$ ;  $r = 0.49$ ), DE intake ( $P < 0.01$ ;  $r = 0.51$ ), milk yield ( $P < 0.01$ ;  $r = 0.65$ ), milk fat yield ( $P < 0.01$ ;  $r = 0.92$ ), and ECM yield ( $P < 0.01$ ;  $r = 0.93$ ). In contrast, energy balance was negatively related to milk yield ( $P < 0.01$ ;  $r = -0.33$ ), milk fat yield ( $P < 0.01$ ;  $r = -0.81$ ), and ECM yield ( $P < 0.01$ ;  $r = -0.72$ ), and positively related to total absorbed FA ( $P < 0.01$ ;  $r = 0.24$ ).

## DISCUSSION

The challenge of meeting nutrient requirements is greater during early lactation than other lactation stages due to an increased risk of negative energy and nutrient balance (NRC, 2001). The enhanced nutrient demand for milk production requires homeorhetic adaptations to support both the increased energy demand of the mammary gland and peripheral tissue metabolism (Bauman and Currie, 1980). Feeding supplemental fat may be a strategy to increase energy intake and reduce negative energy balance, but inconsistent responses to supplemental fat have been observed (e.g., Moallem et al., 2007; Piantoni et al., 2015b; de Souza et al., 2019a). In contrast, research is progressing from feeding traditional animal and plant fats to feeding individual FA and blends of FA, which extends beyond their energy contribution to include potentially structural, metabolic, and physiological effects (Palmquist and Jenkins, 2017). For example, our recent research suggests 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 post-peak cows (de Souza et al., 2018; 2019b). Feeding high levels of C16:0 increased milk fat yield and energy partitioning to milk compared with nonfat control diets and other FA supplements (de Souza and Lock 2018; Western et al., 2020), whereas feeding *cis*-9 C18:1 increased total FA digestibility and nutrient partitioning to body reserves in post-peak cows compared with nonfat supplemented control diets and other FA supplements (de Souza et al., 2018; 2019b). Because metabolic state and stage of lactation play a critical role in energy partitioning, we evaluated the effects of altering the dietary ratio of C16:0 and *cis*-9 C18:1 in supplemental fat blends on nutrient digestibility and metabolic responses of early lactation dairy cows, and production responses are presented in our companion paper (de Souza et al., 2021).

In our study, feeding supplemental fat increased total FA digestibility compared with CON. We varied levels of C16:0 (from 0.36 to 1.57% diet DM) and *cis*-9

C18:1 (from 0.46 to 0.90% diet DM), whereas the other FA were fed at a similar level (Supplemental Table S1, <https://doi.org/10.3168/jds.2020-19312>). To our knowledge, few studies have measured FA digestibility in early lactation cows. Bines et al. (1978) fed increasing levels of tallow in the first 13 wk of lactation and observed a quadratic response in total FA digestibility measured at wk 10 to 12 of lactation. de Souza et al. (2019a) reported that feeding a C16:0-enriched supplement during the immediate postpartum decreased the digestibility of 16-carbon and total FA; however, the difference between treatments for these variables reduced over time. Variable effects on FA digestibility have been observed in post-peak cows; although Rico et al. (2014) reported that feeding a C16:0 supplement had positive effects on 16-carbon and total FA digestibility, other studies with high-producing cows have observed reductions in FA digestibility when feeding similar supplements (de Souza et al., 2017; Rico et al., 2017a). In a recent meta-analysis, Boerman et al. (2015) observed no reduction in FA digestibility when the duodenal flow of C16:0 increased up to 500 g/d, whereas increasing the duodenal flow of C18:0 to the same level reduced FA digestibility. Additionally, 2 recent studies indicated no difference in FA digestibility between control and a C16:0 supplemented diets when the FA supplement contained at least 10% of *cis-9* C18:1 (de Souza et al., 2018; Western et al., 2020). This suggests that although total flow of FA to the duodenum affects FA digestibility (Boerman et al., 2015), the profile of FA entering the duodenum is a critical factor affecting FA digestibility (Doreau and Chilliard, 1997; de Souza et al., 2018).

Additionally, in our study we observed a positive linear effect of increasing dietary *cis-9* C18:1 on 16-carbon, 18-carbon and total FA digestibility. Similarly, in post-peak cows, de Souza et al. (2019b) observed increases in total FA digestibility with increasing level

of *cis-9* C18:1 in supplemental fat (from 10 to 30%) replacing C16:0 (from 80 to 60%) with no interactions with cow production level. Although there are indications that feeding *cis-9* C18:1 improves FA digestibility, the mechanism involved in these changes is not well understood. In ruminants, micelle formation is critical for transporting FA through the aqueous environment to the enterocyte surface (Davis, 1990). Freeman (1969) examined the amphiphilic properties of polar lipids in bile salt solutions and reported that *cis-9* C18:1 had a positive effect on the micellar solubility of C18:0. Additionally, a faster uptake of unsaturated FA compared with saturated FA across the enterocyte plasma membrane and faster re-esterification within the enterocyte has been also proposed as a mechanism explaining differences in digestibility of individual FA (Ockner et al., 1972). Although we increased dietary *cis-9* C18:1 using mainly FA as Ca salts, it is likely that this treatment increased rumen outflow of other 18-carbon FA since it is well established that Ca-salts dissociate to some degree in the rumen (Jenkins and Bridges, 2007). Therefore, it is unclear if these results are exclusively associated with an overall effect of 18-carbon FA or a specific FA altering digestibility. Interestingly, in our study we observed that total absorbed FA was positively related to production outcomes (yields of milk, milk fat, and ECM) and negatively related to plasma NEFA and BW loss. In a recent study with postpeak cows, infusing an emulsifier postruminally increased FA digestibility and also the yields of milk fat and ECM (de Souza et al., 2020). Therefore, these results highlight the importance of FA digestibility on production and metabolic responses of lactating dairy cows.

Typically, fat supplements minimally influence the digestibility of large aggregated fractions, such as DM digestibility, even when the digestibility of total FA differs markedly (Grummer, 1988; Weiss and Wyatt, 2004). However, in our study, we observed that FA-

**Table 3.** Plasma insulin and metabolites for cows fed diets during treatment period (d 1 to 24 postpartum)

Item	Treatment <sup>1</sup>					Contrast <sup>2</sup>			P-value <sup>3</sup>	
	CON	80:10	70:20	60:30	SEM	CON vs. FAT	Linear	Quadratic	Time	Trt × Time
Insulin, $\mu\text{g/L}$	0.26	0.27	0.31	0.31	0.01	0.02	0.07	0.55	0.04	0.21
NEFA, <sup>4</sup> mEq/L	0.72	0.84	0.75	0.67	0.05	0.57	0.03	0.87	<0.01	0.56
BHB, mg/dL	10.6	11.5	13.3	14.8	1.53	0.14	0.10	0.91	0.37	0.15
Albumin, g/dL	3.08	3.27	3.17	3.18	0.06	0.11	0.26	0.48	<0.01	0.96

<sup>1</sup>Treatments were: CON (control; no supplemental fat); 80:10 [1.5% of fatty acid (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); and 60:30 (1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis-9* C18:1).

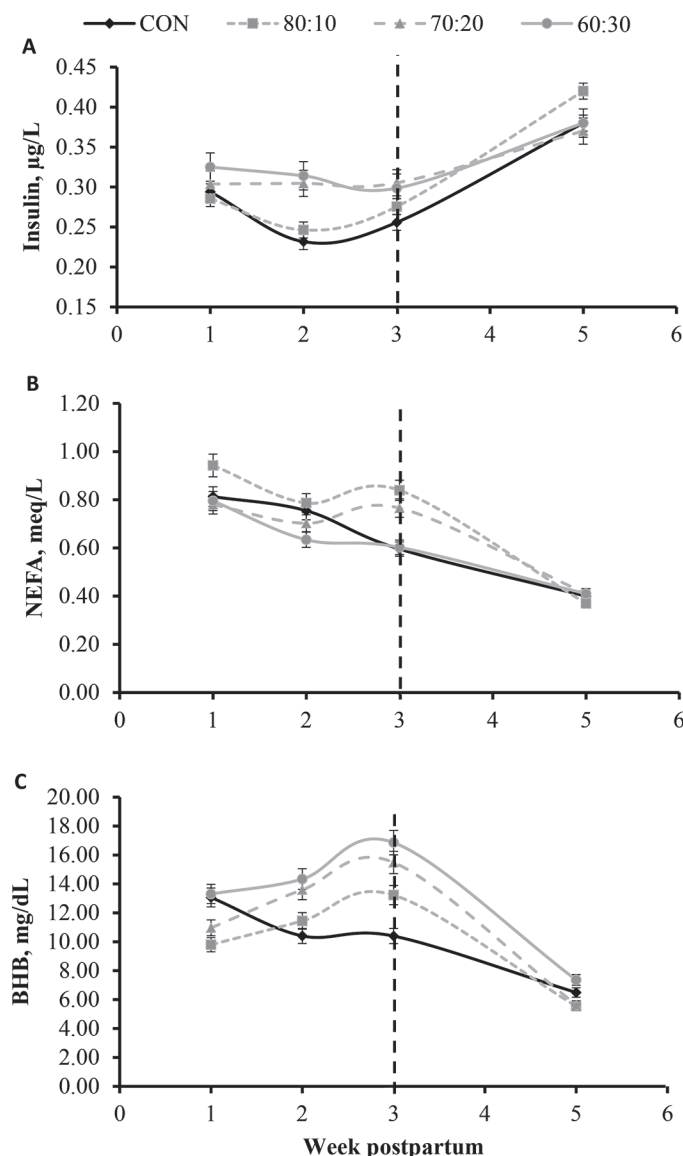
<sup>2</sup>P-values associated with contrasts of treatment effects: CON vs. FAT [control vs. FA-supplemented diets: (80:10 + 70:20 + 60:30)/3]; linear and quadratic effects of *cis-9* C18:1 inclusion in supplemental fat.

<sup>3</sup>Trt = treatment.

<sup>4</sup>Nonesterified fatty acids.



supplemented diets consistently increased both NDF and DM digestibilities compared with CON. Piantoni et al. (2015a) observed that feeding a saturated FA



**Figure 4.** Effects of dietary treatments on (A) plasma insulin, (B) nonesterified fatty acids (NEFA), and (C) BHB over time during the treatment (1–24 DIM) and carryover (25–63 DIM) periods. Data were analyzed separately for the treatment and carryover periods. Diets fed during the treatment period were CON (control diet not supplemented with FA); 80:10 (80% C16:0 + 10% *cis*-9 C18:1); 70:20 (70% C16:0 + 20% *cis*-9 C18:1); and 60:30 (60% C16:0 + 30% *cis*-9 C18:1). 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 plasma insulin ( $P = 0.02$ ). Also, we tended to observe an interaction between treatment and time for BHB ( $P = 0.15$ ) due to FA-supplemented diets increasing BHB compared with CON at wk 3. Increasing *cis*-9 C18:1 in FA treatments linearly decreased plasma NEFA ( $P = 0.03$ ), and tended to linearly increase insulin ( $P = 0.07$ ) and BHB ( $P = 0.10$ ). Error bars indicate SEM.

supplement (C16:0 + C18:0) increased NDF digestibility by 4.0% units in a low forage NDF diet but had no effect in a high forage NDF diet during the immediate postpartum period. de Souza et al. (2019a) reported that feeding a C16:0-enriched supplement during the immediate postpartum increased the digestibility of NDF and DM. With postpeak cows, previous studies have consistently reported that feeding C16:0-enriched supplements increase NDF digestibility compared with nonfat supplemented control diets (Rico et al., 2017a; de Souza and Lock, 2018) and to other FA supplements (de Souza et al., 2018; Western et al., 2020). Feeding C16:0 may increase NDF digestibility due to an increase in retention time driven by an increase in cholecystokinin secretion (Piantoni et al., 2013). Additionally, rumen bacteria typically synthesize C16:0 de novo to produce phosphatidic acid, the precursor for FA components in membranes of *Butyrivibrio* bacteria (Hackmann and Firkins, 2015). However, if dietary C16:0 could be incorporated into rumen bacterial membranes, considerable energy would be spared which may favor bacterial growth (Vlaeminck et al., 2006), potentially increasing NDF digestibility. Therefore, the results of our study with early-lactation cows agree with previous findings in indicating a positive effect of feeding C16:0 on fiber digestibility. Also, in our study, we observed a linear positive effect of increasing dietary *cis*-9 C18:1 on DM and NDF digestibility. Furthermore, the difference in DM digestibility between the CON and 60:30 treatments tended to increase over time. Feed intake and nutrient digestibility are often negatively related (NRC, 2001). However, in our trial the 60:30 treatment increased both DMI and nutrient digestibility compared with the CON treatment. The NDF digestibility response to dietary *cis*-9 C18:1 have been inconsistent across studies. For instance, Lopes et al. (2017) did not observe differences in NDF digestibility when replacing conventional soybean with *cis*-9 C18:1 enriched soybean, whereas a recent in vitro study reported decreased fiber digestibility when *cis*-9 C18:1 as a free FA was added to continuous culture fermenters (Sears et al., 2019). In contrast, de Souza et al. (2019b) reported no treatment differences for digestibilities of DM and NDF when feeding different ratios of C16:0 and *cis*-9 C18:1 for post-peak cows. Therefore, other factors such as level of supplementation, form of delivery of FA, and potential interactions with other FA and dietary factors may influence the response on nutrient digestibility to dietary *cis*-9 C18:1.

Increasing energy intake and improving energy balance is one of the main goals of nutritional management during early lactation. In our study, we observed positive correlations between DE intake and yields of milk and ECM and a negative relationship between DE

**Table 4.** Plasma insulin and metabolites for cows fed diets during the carryover period

Item	Treatment <sup>1</sup>				SEM	Contrast <sup>2</sup>		
	CON	80:10	70:20	60:30		CON vs. FAT	Linear	Quadratic
Insulin, $\mu\text{g/L}$	0.38	0.42	0.37	0.38	0.02	0.81	0.38	0.58
NEFA, <sup>3</sup> mEq/L	0.40	0.37	0.41	0.41	0.07	0.76	0.49	0.68
BHB, mg/dL	6.49	5.54	5.64	7.36	0.86	0.75	0.13	0.44
Albumin, g/dL	3.34	3.41	3.41	3.39	0.06	0.43	0.80	0.96

<sup>1</sup>Treatments were: CON (control; no supplemental fat); 80:10 [1.5% of fatty acid (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); and 60:30 (1.5% of FA supplement blend to provide ~60% C16:0 and 30% *cis*-9 C18:1).

<sup>2</sup>*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.

<sup>3</sup>Nonesterified fatty acids.

intake and BW loss and plasma NEFA concentration. Overall, FA-supplemented diets increased energy intake including DE, ME, and  $\text{NE}_L$  compared with CON. Previous studies feeding FA supplements around parturition have reported inconsistent results regarding energy intake. Reasons for these inconsistent results regarding the effect of supplemental fat may include different methods to calculate or predict energy intake, duration of the supplementation period, level of supplemental fat in the diet, and most likely the FA profile of supplemental fat. Piantoni et al. (2015b) reported that cows immediately postpartum had increased energy intake (+ 4.2 Mcal of  $\text{NE}_L/\text{d}$ ) when fed a saturated FA supplement (C16:0 + C18:0) regardless of dietary forage NDF level partially due to the high DMI in FA-supplemented diets (+1.4 kg of DMI/d). de Souza et al. (2019a) reported that feeding a C16:0-enriched supplement during the immediate postpartum increased energy intake (+ 3.7 Mcal of  $\text{NE}_L/\text{d}$ ) partially because of the positive effect of this FA supplement on NDF digestibility. In contrast, Moallem et al. (2007) fed a saturated FA supplement (C16:0 + C18:0) during the pre- and postpartum periods and reported that the supplement did not affect predicted energy intake compared with a diet with no supplemental fat. In our study, we observed a positive linear effect of increasing dietary *cis*-9 C18:1 on energy intake, which was associated with a linear increase in feed intake and nutrient digestibility (NDF and FA) as dietary *cis*-9 C18:1 increased. As discussed by de Souza et al. (2019a), considering the potential effect that individual FA may have on feed intake and nutrient digestibility, measuring energy intake rather than predicting it from dietary composition is vital for determining energy intake and energy balance.

In our study, the 80:10 treatment increased milk energy output and the efficiency of energy utilization to milk compared with other treatments because of the increase in milk fat yield (de Souza et al., 2021); however, it also increased plasma NEFA and negative energy balance. Similarly, in early lactation cows, we previously

observed that feeding a C16:0-enriched supplement increased ECM and milk energy output, but decreased BW, BCS, and increased NEFA and negative energy balance when fed during the first 24 d after calving (de Souza and Lock, 2019; de Souza et al., 2019a). In contrast, most studies feeding C16:0 supplements to postpeak cows (fed at 1.5 to 2.0% diet DM) have indicated no changes in BW and BCS compared with control diets (Rico et al., 2017a; de Souza and Lock, 2018; Western et al., 2020). In postpeak cows, Mathews et al. (2016) observed a decrease in glucose-stimulated NEFA disappearance in cows fed C16:0, suggesting the possibility of localized adipose tissue insulin resistance with prolonged C16:0 supplementation. Also, feeding C16:0 in early lactation rapidly increased circulating ceramide, especially C24:0-ceramide (Davis et al., 2017), which is inversely associated with glucose clearance rates following an insulin challenge postpartum (Rico et al., 2017b). Because the development of insulin resistance in adipose and skeletal muscle tissues enables the dairy cow to partition nutrients toward the mammary gland during early lactation (Bell, 1995; Bell and Bauman, 1997), it is possible that the change in energy partitioning to milk at the expense of body reserves in the immediate postpartum period in the 80:10 treatment may be partially related to changes in insulin resistance. Although lipolysis ensures an adequate supply of energy substrates to tissues around parturition, intense and protracted lipolysis predisposes cows to inflammatory and metabolic diseases by limiting the capacity of adipose tissue for energy buffering and contributing to chronically increased plasma NEFA (Contreras et al., 2017). Plasma NEFA concentrations greater than 0.7 mmol/L in the immediate postpartum period have been described as a risk factor for the development of clinical diseases postpartum (i.e., clinical ketosis, metritis, displaced abomasum), impaired reproduction in the subsequent breeding period, and early culling from the herd (Ospina et al., 2013; Ribeiro et al., 2013). We observed that during the first week after calving the

**Table 5.** Correlation coefficients between production variables, plasma metabolites, and energy parameters for cows fed treatment diets (1–24 DIM)

Item <sup>1</sup>	Milk yield, kg	DMI, kg	Milk fat yield, kg	Milk fat, %	ECM, kg	BW loss, kg/d	Insulin, µg/L	Albumin, g/dL	NEFA, mEq/L	BHB, mg/dL	Total FA absorbed, g	DE, Mcal	Milk energy output, Mcal	Energy balance, Mcal
Milk yield, kg	1.00	0.55 <sup>2</sup>	0.41	-0.30	0.48	-0.01	-0.06	0.41	-0.08	0.19	0.42	0.60	0.65	-0.33
DMI, kg	<0.01 <sup>3</sup>	1.00	<0.01	<0.01	<0.01	0.91	0.45	<0.01	0.31	0.02	<0.01	<0.01	<0.01	<0.01
Milk fat yield, kg	<0.01	0.30	1.00	0.03	<0.01	0.26	0.16	0.29	-0.27	0.18	0.52	0.79	0.43	-0.08
Milk fat, %	0.29	0.81	1.00	1.00	0.81	-0.04	0.06	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	0.33
ECM, kg	<0.01	0.04	0.64	<0.01	1.00	0.64	-0.01	0.08	-0.09	0.23	0.35	0.29	0.92	-0.81
BW loss, kg/d	0.07	0.65	0.41	1.00	1.00	-0.07	0.86	0.30	0.26	0.01	<0.01	<0.01	<0.01	<0.01
Insulin, µg/L	0.02	0.85	0.18	0.02	0.93	0.01	-0.02	0.10	0.10	0.27	0.27	0.31	0.08	-0.31
Albumin, g/dL	0.04	0.85	0.02	0.04	1.00	0.85	0.02	0.20	-0.14	<0.01	0.37	0.35	0.93	<0.01
NEFA, mEq/L	0.01	0.18	0.09	0.01	1.00	0.02	0.18	-0.16	0.23	0.10	<0.01	0.01	0.61	<0.01
BHB, mg/dL	0.07	0.02	0.04	0.04	1.00	0.02	1.00	-0.04	<0.01	-0.14	-0.14	0.22	0.01	0.12
Total FA absorbed, <sup>3</sup> g	0.07	0.88	0.29	0.01	1.00	0.07	0.07	0.59	0.01	0.07	<0.01	0.36	0.27	0.54
DE, Mcal	0.42	1.00	0.42	1.00	1.00	0.42	0.42	1.00	1.00	0.42	<0.01	0.36	<0.01	-0.02
Milk energy output, Mcal	0.12	0.16	0.16	1.00	1.00	0.12	0.16	1.00	1.00	0.12	-0.18	-0.25	-0.16	0.08
Energy balance, Mcal	0.06	0.03	0.03	1.00	1.00	0.06	0.03	1.00	1.00	0.06	0.03	<0.01	0.06	0.32
	0.05	0.70	0.70	1.00	1.00	0.05	0.70	1.00	1.00	0.05	0.03	-0.19	0.05	-0.12
	0.24	0.73	0.73	1.00	1.00	0.24	0.73	1.00	1.00	0.24	0.73	0.02	0.55	0.16
	<0.01	<0.01	<0.01	1.00	1.00	<0.01	<0.01	1.00	1.00	<0.01	<0.01	<0.01	<0.01	<0.01
	0.02	1.00	1.00	1.00	1.00	0.02	1.00	1.00	1.00	0.02	1.00	1.00	0.51	0.78
	0.78	0.78	0.78	1.00	1.00	0.78	0.78	1.00	1.00	0.78	0.78	0.78	1.00	0.78
	<0.01	<0.01	<0.01	1.00	1.00	<0.01	<0.01	1.00	1.00	<0.01	<0.01	<0.01	<0.01	<0.01
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

<sup>1</sup>NEFA = nonesterified fatty acids; FA = fatty acids; DE = digestible energy.<sup>2</sup>The Pearson correlation coefficient of the linear relationship between 2 variables. The value for each cow per week was used as independent variables.<sup>3</sup>The *P*-value associated with the linear relationship between 2 variables.

number of cows with NEFA concentrations in serum greater than 0.7 mmol/L was similar between CON and 80:10 (54 and 61% for CON and 80:10, respectively). Although the 80:10 treatment increased markers of lipolysis in the immediate postpartum, we observed a pronounced positive carryover effect of this treatment on milk yield (de Souza et al., 2021). Further studies are needed to understand the mechanism by which C16:0 supplementation increases milk energy output at the expense of body reserves in the immediate postpartum period, and the possible effects of greater BW and BCS losses on health and reproduction of dairy cows.

One of our hypotheses in this study was that increasing the amount of *cis*-9 C18:1 in supplemental fat would reduce milk energy output and improve energy balance in early lactation. Interestingly, we observed that increasing dietary *cis*-9 C18:1 improved energy balance by decreasing BW and BCS losses compared with the 80:10 treatment. The differences in nutrient partitioning are probably driven by insulin, as we observed that increasing *cis*-9 C18:1 increased plasma insulin concentration. Similarly, previous studies indicated that feeding *cis*-9 C18:1 not only increased BW gain in postpeak cows but also plasma insulin compared with non-fat-supplemented diets and other FA supplements (de Souza et al., 2018, 2019b). Although to our knowledge this has not been studied in cows, previous studies using rats have observed that *cis*-9 C18:1 stimulated insulin secretion from pancreatic  $\beta$ -cells (Itoh et al., 2003; Fujiwara et al., 2005). Elevated insulin concentrations would reduce plasma NEFA through inhibiting lipolysis or increasing lipogenesis (Vernon, 2005). In addition, increased concentrations of plasma triglycerides (TAG) could result from increased absorption of dietary FA increasing the supply of TAG-rich lipoproteins available in circulation. As a result, increases in insulin secretion could partition circulating TAG into other tissues and reduce lipolysis from adipose tissues. Furthermore, Yanting et al. (2018) reported that *cis*-9 C18:1 increased adipocyte number and size through enhancing adipogenic commitment and lipogenesis compared with saturated FA (C14:0, C16:0, and C18:0). Also, the latter authors reported that in mature adipocytes treated with FA, the lipid content in adipocytes was affected by FA profile and ranked as *cis*-9 C18:1 > *cis*-9 *cis*-12 C18:2 > C14:0 > C18:0 > C16:0. Although we observed that feeding *cis*-9 C18:1 decreased plasma NEFA, a tendency for a linear increase in BHB was observed. Importantly, BHB does not only originate from ketogenesis in the liver from FA but also from conversion in the rumen epithelial cell of butyrate to BHB (Weigand et al., 1972). Infusion of butyrate in the rumen has been positively related to DMI and plasma

BHB (Herrick et al., 2018). Therefore, in our study, the increase in plasma BHB when feeding *cis*-9 C18:1 may be related to the greater DMI observed. Of note in our study, increasing *cis*-9 C18:1 in FA treatments tended to reduce body reserve mobilization despite the increase in milk energy output indicating that this could be a strategy to improve cow energy status and health without decreasing performance. Further research is needed to determine the mechanism by which *cis*-9 C18:1 may alter energy partitioning in early lactation cows.

## CONCLUSIONS

Our results indicate that feeding FA supplements containing C16:0 and *cis*-9 C18:1 during the immediate postpartum period increased nutrient digestibility, energy intake, and milk energy output compared with a non-fat-supplemented control diet. Increasing dietary C16:0 increased energy intake, milk energy output, but also increased markers of body fat mobilization and negative energy balance during the immediate postpartum. Increasing dietary *cis*-9 C18:1 increased energy intake, reduced markers of body fat mobilization and improved energy balance during the immediate postpartum.

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## REFERENCES

- Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its



- application to ruminants. *J. Anim. Sci.* 87:3317–3334. <https://doi.org/10.2527/jas.2009-1779>.
- Allen, M. S., and P. Piantoni. 2013. Metabolic control of feed intake: Implications for metabolic disease of fresh cows. *Vet. Clin. North Am. Food Anim. Pract.* 29:279–297. <https://doi.org/10.1016/j.cvfa.2013.04.001>.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529.
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804–2819. <https://doi.org/10.2527/1995.7392804x>.
- Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. *J. Mammary Gland Biol. Neoplasia* 2:265–278. <https://doi.org/10.1023/A:1026336505343>.
- Bines, J. A., P. E. Brumby, J. E. Storry, R. J. Fulford, and G. D. Braithwaite. 1978. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cows during early lactation. *J. Agric. Sci.* 91:135–150. <https://doi.org/10.1017/S0021859600056690>.
- Boerman, J. P., J. de Souza, and A. L. Lock. 2017. Milk production and nutrient digestibility responses to increasing levels of stearic acid supplementation of dairy cows. *J. Dairy Sci.* 100:2729–2738. <https://doi.org/10.3168/jds.2016-12101>.
- Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. *J. Dairy Sci.* 98:8889–8903. <https://doi.org/10.3168/jds.2015-9592>.
- Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* 98:6631–6650. <https://doi.org/10.3168/jds.2015-9683>.
- Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyeen. 1986. Predicting the digestibility of different diets with internal markers: Evaluation of four potential markers. *J. Anim. Sci.* 63:1476–1483. <https://doi.org/10.2527/jas1986.6351476x>.
- Contreras, G. A., C. Strieder-Barboza, and W. Raphael. 2017. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. *J. Anim. Sci. Biotechnol.* 8:41. <https://doi.org/10.1186/s40104-017-0174-4>.
- Davis, A. N., Z. C. Phipps, Q. Zeng, J. de Souza, J. E. Rico, A. L. Lock, and J. W. McFadden. 2017. Palmitic acid feeding increases plasma ceramide concentrations in Holstein dairy cows during early lactation. *J. Dairy Sci.* 100(Suppl. 2):101 (Abstr.)
- Davis, C. L. 1990. Fats in Animal Feeds. Barnaby Inc., Sycamore, IL.
- De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. *Vet. Clin. North Am. Food Anim. Pract.* 29:299–322. <https://doi.org/10.1016/j.cvfa.2013.04.002>.
- de Souza, J., F. Batistel, and F. A. Santos. 2017. Effect of sources of calcium salts of fatty acids on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows. *J. Dairy Sci.* 100:1072–1085. <https://doi.org/10.3168/jds.2016-11636>.
- de Souza, J., and A. L. Lock. 2018. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. *J. Dairy Sci.* 101:3044–3056. <https://doi.org/10.3168/jds.2017-13946>.
- de Souza, J., and A. L. Lock. 2019. Effects of timing of palmitic acid supplementation on production responses of early-lactation dairy cows. *J. Dairy Sci.* 102:260–273. <https://doi.org/10.3168/jds.2018-14976>.
- de Souza, J., C. L. Preseault, and A. L. Lock. 2018. Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. *J. Dairy Sci.* 101:172–185. <https://doi.org/10.3168/jds.2017-13460>.
- de Souza, J., C. M. Prom, and A. L. Lock. 2021. Altering the ratio of dietary palmitic and oleic acids impacts production responses during the immediate postpartum and carryover periods in dairy cows. *J. Dairy Sci.* 104:2896–2909. <https://doi.org/10.3168/jds.2020-19311>.
- de Souza, J., N. R. St-Pierre, and A. L. Lock. 2019b. Altering the ratio of dietary C16:0 and *cis*-9 C18:1 interacts with production level in dairy cows: effects on production responses and energy partitioning. *J. Dairy Sci.* 102:9842–9856. <https://doi.org/10.3168/jds.2019-16374>.
- de Souza, J., C. Strieder-Barboza, G. A. Contreras, and A. L. Lock. 2019a. Effects of timing of palmitic acid supplementation during early lactation on nutrient digestibility, energy balance, and metabolism of dairy cows. *J. Dairy Sci.* 102:274–287. <https://doi.org/10.3168/jds.2018-14977>.
- de Souza, J., M. Western, and A. L. Lock. 2020. Abomasal infusion of an exogenous emulsifier improves fatty acid digestibility and milk fat yield of lactating dairy cows. *J. Dairy Sci.* 103:6167–6177. <https://doi.org/10.3168/jds.2020-18239>.
- Doreau, M., and Y. Chilliard. 1997. Digestion and metabolism of dietary fat in farm animals. *Br. J. Nutr.* 78(Suppl. 1):S15–S35. <https://doi.org/10.1079/BJN19970132>.
- Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. *Br. J. Nutr.* 23:249–263. <https://doi.org/10.1079/BJN19690032>.
- Fujiwara, K., F. Maekawa, and T. Yada. 2005. Oleic acid interacts with GPR40 to induce Ca<sup>2+</sup> signaling in rat islet beta-cells: mediation by PLC and L-type Ca<sup>2+</sup> channel and link to insulin release. *Am. J. Physiol. Endocrinol. Metab.* 289:E670–E677. <https://doi.org/10.1152/ajpendo.00035.2005>.
- Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agricultural Handbook no. 379. ARS-USDA, Washington, DC.
- González, F. D., R. Muiño, V. Pereira, R. Campos, and J. L. Benedito. 2011. Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. *J. Vet. Sci.* 12:251–255. <https://doi.org/10.4142/jvs.2011.12.3.251>.
- Grummer, R. R. 1988. Influence of prilled fat and calcium salts of palm oil fatty acids on ruminant fermentation and nutrient digestibility. *J. Dairy Sci.* 71:117–123. [https://doi.org/10.3168/jds.S0022-0302\(88\)79532-6](https://doi.org/10.3168/jds.S0022-0302(88)79532-6).
- Hackmann, T. J., and J. L. Firkins. 2015. Electron transport phosphorylation in rumen butyrovibrios: Unprecedented ATP yield for glucose fermentation to butyrate. *Front. Microbiol.* 6:622. <https://doi.org/10.3389/fmicb.2015.00622>.
- Harvatiné, K. J., and M. S. Allen. 2006. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. *J. Dairy Sci.* 89:1081–1091. [https://doi.org/10.3168/jds.S0022-0302\(06\)72176-2](https://doi.org/10.3168/jds.S0022-0302(06)72176-2).
- Herrick, K. J., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, S. D. Ranathunga, J. L. Anderson, S. C. Moreland, and J. E. van Eys. 2018. Infusion of butyrate affects plasma glucose, butyrate, and  $\beta$ -hydroxybutyrate but not plasma insulin in lactating dairy cows. *J. Dairy Sci.* 101:3524–3536. <https://doi.org/10.3168/jds.2017-13842>.
- Itoh, Y., Y. Kawamata, M. Harada, M. Kobayashi, R. Fujii, S. Fukusumi, K. Ogi, M. Hosoya, Y. Tanaka, H. Uejima, H. Tanaka, M. Maruyama, R. Satoh, S. Okubo, H. Kizawa, H. Komatsu, F. Matsumura, Y. Noguchi, T. Shinohara, S. Hinuma, Y. Fujisawa, and M. Fujino. 2003. Free fatty acids regulate insulin secretion from pancreatic  $\beta$  cells through GPR40. *Nature* 422:173–176. <https://doi.org/10.1038/nature01478>.
- Jenkins, T. C., and W. C. Bridges Jr. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. *Eur. J. Lipid Sci. Technol.* 109:778–789. <https://doi.org/10.1002/ejlt.200700022>.
- Lopes, J. C., M. T. Harper, F. Giallongo, J. Oh, L. Smith, A. M. Ortega-Perez, S. A. Harper, A. Melgar, D. M. Kniffen, R. A. Fabin, and A. N. Hristov. 2017. Effect of high-oleic-acid soybeans on production performance, milk fatty acid composition, and enteric methane emission in dairy cows. *J. Dairy Sci.* 100:1122–1135. <https://doi.org/10.3168/jds.2016-11911>.
- Mathews, A. T., J. E. Rico, N. T. Sprenkle, A. L. Lock, and J. W. McFadden. 2016. Increasing palmitic acid intake enhances milk production and prevents glucose-stimulated fatty acid disappearance without modifying systemic glucose tolerance in mid-lacta-

- tion dairy cows. *J. Dairy Sci.* 99:8802–8816. <https://doi.org/10.3168/jds.2016-11295>.
- Moallem, U., M. Katz, A. Arieli, and H. Lehrer. 2007. Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows. *J. Dairy Sci.* 90:3846–3856. <https://doi.org/10.3168/jds.2007-0092>.
- NRC (National Research Council). 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academies Press, Washington, DC.
- Ockner, R. K., J. P. Pittman, and J. L. Yager. 1972. Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids. *Gastroenterology* 62:981–992. [https://doi.org/10.1016/S0016-5085\(72\)80115-X](https://doi.org/10.1016/S0016-5085(72)80115-X).
- Ospina, P. A., J. A. McArt, T. R. Overton, T. Stokol, and D. V. Nydam. 2013. Using nonesterified fatty acids and  $\beta$ -hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. *Vet. Clin. North Am. Food Anim. Pract.* 29:387–412. <https://doi.org/10.1016/j.cvfa.2013.04.003>.
- Overton, T. R., J. A. A. McArt, and D. V. Nydam. 2017. A 100-year review: Metabolic health indicators and management of dairy cattle. *J. Dairy Sci.* 100:10398–10417. <https://doi.org/10.3168/jds.2017-13054>.
- Palmquist, D. L., and T. C. Jenkins. 2017. A 100-Year Review: Fat feeding of dairy cows. *J. Dairy Sci.* 100:10061–10077. <https://doi.org/10.3168/jds.2017-12924>.
- Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. *J. Dairy Sci.* 96:7143–7154. <https://doi.org/10.3168/jds.2013-6680>.
- Piantoni, P., A. L. Lock, and M. S. Allen. 2015a. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. *J. Dairy Sci.* 98:3309–3322. <https://doi.org/10.3168/jds.2014-8798>.
- Piantoni, P., A. L. Lock, and M. S. Allen. 2015b. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum period in Holstein cows: Energy balance and metabolism. *J. Dairy Sci.* 98:3323–3334. <https://doi.org/10.3168/jds.2014-8799>.
- Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. *J. Dairy Sci.* 96:5682–5697. <https://doi.org/10.3168/jds.2012-6335>.
- Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. *J. Dairy Sci.* 97:3739–3751. <https://doi.org/10.3168/jds.2013-7341>.
- Rico, J. E., J. de Souza, M. S. Allen, and A. L. Lock. 2017a. Nutrient digestibility and milk production responses to increasing levels of palmitic acid supplementation vary in cows receiving diets with or without whole cottonseed. *J. Anim. Sci.* 95:436–446.
- Rico, J. E., S. Saed Samii, A. T. Mathews, J. Lovett, N. J. Haughey, and J. W. McFadden. 2017b. Temporal changes in sphingolipids and systemic insulin sensitivity during the transition from gestation to lactation. *PLoS One* 12:e0176787. <https://doi.org/10.1371/journal.pone.0176787>.
- Sears, A., J. de Souza, B. A. Wenner, and F. Batistel. 2019. Palmitic, stearic, and oleic acids differently alter NDF digestibility in a continuous culture system. *J. Dairy Sci.* 102(Suppl. 1):85 (Abstr.).
- Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity. *J. Dairy Sci.* 99:4967–4982. <https://doi.org/10.3168/jds.2015-10354>.
- Sordillo, L. M., G. A. Contreras, and S. L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows. *Anim. Health Res. Rev.* 10:53–63. <https://doi.org/10.1017/S1466252309990016>.
- Vernon, R. G. 2005. Lipid metabolism during lactation: A review of adipose tissue-liver interactions and the development of fatty liver. *J. Dairy Res.* 72:460–469. <https://doi.org/10.1017/S0022029905001299>.
- Vlaeminck, B., V. Fievez, A. J. R. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Anim. Feed Sci. Technol.* 131:389–417. <https://doi.org/10.1016/j.anifeedsci.2006.06.017>.
- Weigand, E., J. W. Young, and A. D. McGilliard. 1972. Extent of butyrate metabolism by bovine rumenoreticulum epithelium and the relationship to absorption rate. *J. Dairy Sci.* 55:589–597. [https://doi.org/10.3168/jds.S0022-0302\(72\)85541-3](https://doi.org/10.3168/jds.S0022-0302(72)85541-3).
- Weiss, W. P., and D. J. Wyatt. 2004. Digestible energy values of diets with different fat supplements when fed to lactating dairy cows. *J. Dairy Sci.* 87:1446–1454. [https://doi.org/10.3168/jds.S0022-0302\(04\)73295-6](https://doi.org/10.3168/jds.S0022-0302(04)73295-6).
- Western, M. M., J. de Souza, and A. L. Lock. 2020. Effects of commercially available palmitic and stearic acid supplements on nutrient digestibility and production responses of lactating dairy cows. *J. Dairy Sci.* 103:5131–5142. <https://doi.org/10.3168/jds.2019-17242>.
- Yanting, C., Q. Y. Yang, G. L. Ma, M. Du, J. H. Harrison, and E. Block. 2018. Dose- and type-dependent effects of long-chain fatty acids on adipogenesis and lipogenesis of bovine adipocytes. *J. Dairy Sci.* 101:1601–1615. <https://doi.org/10.3168/jds.2017-13312>.
- Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. *J. Dairy Sci.* 96:5656–5669. <https://doi.org/10.3168/jds.2012-6142>.