Effect of sources of calcium salts of fatty acids on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows

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ABSTRACT

The objective of our study was to investigate the effects of sources of calcium salts of fatty acids (FA) on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows. Treatment diets were offered from 3 to 16 wk postpartum (the treatment period), in which all cows grazed elephantgrass (*Pennisetum purpureum* ‘Cameroon’) and treatments were added to a concentrate supplement. The treatments were (1) control (concentrate without supplemental fat); (2) concentrate with calcium salts of soybean FA (CSSO); and (3) concentrate with calcium salts of palm FA (CSPO). From 17 to 42 wk postpartum (the carryover period), all cows received a common diet fed as a total mixed ration. During the treatment period, CSPO increased milk yield, milk fat yield, 3.5% fat-corrected milk, energy-corrected milk, and cumulative milk yield compared with control and CSSO. Treatment CSSO increased the yield of milk but did not affect 3.5% fat-corrected milk or energy-corrected compared with control. Also, CSSO decreased milk fat yield, dry matter intake, neutral detergent fiber digestibility, and body weight and body condition loss. Compared with control, both CSSO and CSPO increased feed efficiency (3.5% fat-corrected milk:dry matter intake), and CSPO increased feed efficiency compared with CSSO. When considering energy partitioning (as % energy intake), CSPO increased energy partitioning toward milk and increased energy mobilized from body reserves compared with control and CSSO. Furthermore, CSSO tended to reduce the mobilization of energy from body reserves compared with control. In the carryover period, no differences in milk composition were observed among treatments. A treatment by time interaction was observed during the carryover period for milk yield because cows on CSPO

maintained higher production compared with control and CSSO cows until 30 wk postpartum; CSSO had a lower carryover effect sustaining higher milk yield compared with control until 25 wk postpartum. In conclusion, supplementation with CSPO was an effective strategy to increase energy intake and yields of milk and milk solids and it had a greater carryover effect. Supplementation with CSSO resulted in lower mobilization of reserves and less variation in body weight and body condition throughout lactation.

Key words: calcium salts, carryover effect, palm fatty acids, soybean fatty acids, tropical pasture

INTRODUCTION

Dairy production in many countries is mainly based on grazing systems. Seasonal variations in pasture availability and nutritive value (Roche et al., 2009a) and the need to meet the nutritional requirements of high-producing cows (Peyraud and Delagarde, 2013) are the main limitations of grazing systems. Although recommended grazing management practices have considerably improved the nutritive value of tropical grasses (Da Silva et al., 2013) and animal performance (Voltolini et al., 2010), sward structure, NDF content, and low particle fragility of these plants may still limit forage intake and performance (Santos et al., 2014). Energy intake is likely the major restriction to better performance in pasture-based systems (Hills et al., 2015).

During early lactation, dairy cows experience a period of negative energy balance because they cannot consume enough dietary energy to match their requirements for milk production (NRC, 2001). Under grazing conditions, this negative energy balance may even be greater as the consequence of a lower DMI of cows compared with a TMR diet (Muller and Fales, 1998), and a higher requirement for maintenance because of greater physical activity (Dohme-Meier et al., 2014). Feeding concentrate based on cereal grains is the most common strategy used to increase energy intake in pasture-based

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systems (Bargo et al., 2003). However, slug feeding of large amounts of starch-based supplements may result in decreased ruminal pH (Wales and Doyle 2003), NDF digestibility (Wales et al., 2004), total DMI (Auldist et al., 2013), and milk production responses (Doyle et al., 2005). In this context, supplemental fat can be used to increase the energy density of diets and replace, at least in part, cereal grains. Fat supplementation has been shown to increase milk production in housed systems with cows receiving TMR (Rabiee et al., 2012). However, the potential response of fat supplementation in pasture-based diets is poorly described (Schroeder et al., 2004), especially with cows grazing on tropical pastures. Moreover, most of the studies conducted in grazing cows used fat supplements with low digestibility (i.e., hydrogenated tallow, hydrogenated palm oil), which may limit the production response to the supplemental fat (Weiss and Wyatt, 2004). This suggests that production responses of grazing cows could be positively affected by the addition of fat supplements with higher digestibility. Also, the fatty acid (FA) profile of a fat supplement is likely the primary factor affecting production responses to it (Lock and de Souza, 2016). Harvatine and Allen (2006) observed that supplementation with a saturated prilled fat did not affect production responses, whereas feeding a mostly unsaturated calcium salts supplement in the diet linearly increased BW gain and reduced milk energy output. Therefore, determining the response to sources of fat with different FA profiles is of particular importance. Furthermore, Vilela et al. (2002) suggested a potential positive carryover effect of supplementing grazing dairy cows with calcium salts of FA in early lactation. The carryover effect contributes to the profitability of any supplementation strategy and is crucial to predicting the total response to supplements (Roche et al., 2013).

The objective of our study was to investigate the effects of sources of calcium salts of FA on production, nutrient digestibility, energy balance, and carryover effects of early lactation dairy cows grazing on tropical pasture. We postulated that high-producing early lactation dairy cows grazing on tropical pastures are in negative energy balance and that the addition of fat supplements in the diet could be advantageous with a potentially positive carryover effect. We also hypothesized that the production responses would be affected by the FA profile of the fat supplement due to the effect of the degree of saturation on ruminal and postruminal nutrient digestibility and energy partitioning.

MATERIALS AND METHODS

The study was conducted in Piracicaba, São Paulo, Brazil (22.7°S, 47.6°E and 546 m altitude). The Ethical

Committee for Animal Research of the University of São Paulo–Piracicaba approved all procedures involving animals.

Animals and Treatments

Thirty-six multiparous dairy cows (Jersey × Holstein), with (mean ± SD) 492 ± 44 kg of BW and 19 ± 2.1 DIM were used in a randomized complete block design. Cows were blocked by BCS, parity, and previous lactation 305-d milk production. Treatment diets were offered from 3 to 16 wk postpartum (treatment period). The treatments were (1) control (concentrate without supplemental fat); (2) concentrate with calcium salts of soybean FA (CSSO; 400 g/d; Megalac-E, Química Geral do Nordeste, Rio de Janeiro, Brazil); and (3) concentrate with calcium salts of palm FA (CSPO; 400 g/d, Magnapac, Tectron Inc., Toledo, Parana, Brazil). This level of fat supplementation provided 1.9% of FA on diet DM assuming an intake of 18 kg/d and 85% of FA of the calcium salts supplement (NRC, 2001). From 17 to 42 wk postpartum (carryover period), all cows received a common diet fed as TMR. All diets were formulated to meet predicted requirements according to NRC (2001).

During the treatment period, all cows were rotationally grazed elephantgrass (*Pennisetum purpureum* ‘Cameroon’) as one herd, and the treatments were added to a concentrate supplement (8 kg of DM/d). The treatments were fed individually in 2 equal feedings at 0330 and 1430 h before milking. The ingredients and chemical composition of the concentrate mixes used are shown in Table 1. Distance from pastures to the milking parlor averaged 0.4 km (ranging between 0.2 and 0.7 km). During the treatment period, the average temperature was 25.2 ± 1.3°C, relative humidity 76.8 ± 2.0%, and rainfall 200 ± 15.5 mm.

The 5.0 ha of pasture used in this experiment was fertilized with 60 kg of N/ha after each grazing. Experimental cows were allocated to a new paddock of 2,000 m² every day, after evening milkings, with free access to natural shade and fresh water. Elephantgrass was managed in a rotational grazing system based on canopy height, with 103 cm being the entry height target (Voltolini et al., 2010). Cows grazed the canopy down to an average of 62.8 ± 2.2 cm (Table 2). This procedure was adopted according to Fonseca et al. (2013), which recommended that, to avoid a reduction in short-term forage intake by sward structure, cattle should graze the canopy down to a maximum of 40% of the entry height. Subsequently, a group of dry cows and heifers were used to graze down the pasture to final postgrazed height (45 ± 5.0 cm). The average grazing interval was 21.1 ± 2.4 d.

Table 1. Ingredients and nutritional composition (% of DM) of experimental concentrates, forage, and carryover diet

Item	Treatment period				Carryover diet
	Forage	Concentrate ¹			
		Control	CSSO	CSPO	
Ingredient					
Corn silage		—	—	—	63.4
Ground corn		82.5	78.2	78.2	20.3
Soybean meal		12.0	12.0	12.0	13.3
Fat supplement ²		—	4.90	4.90	—
Urea		0.10	0.30	0.30	0.84
Limestone		1.40	0.60	0.60	—
Mineral and vitamin mix ³		4.0	4.0	4.0	2.20
Chemical composition					
DM	19.8	88.8	89.4	89.3	53.9
CP	18.3	14.5	14.4	14.4	16.6
NDF	54.2	9.30	8.80	8.80	37.1
ADF	37.5	3.31	3.22	3.23	17.3
Lignin	3.21	0.42	0.41	0.42	2.32
Ash	11.2	6.82	7.22	7.22	5.61
Total FA	3.22	2.71	6.60	6.60	2.0
16:0	0.52	0.36	1.01	2.22	0.31
18:0	0.10	0.06	0.24	0.23	0.05
18:1 <i>cis</i> -9	0.08	0.64	1.45	1.95	0.40
18:2 <i>cis</i> -9, <i>cis</i> -12	0.46	1.24	3.07	1.19	0.94
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	1.76	0.05	0.18	0.06	0.11

¹Concentrates fed during treatment period were (1) control (concentrate without supplemental fat); (2) concentrate with calcium salts of soybean fatty acids (CSSO); and (3) concentrate with calcium salts of palm fatty acids (CSPO).

²Fat supplements were (1) calcium salts of fatty acids (CSSO; Megalac-E, Química Geral do Nordeste, Rio de Janeiro, Brazil) 85.0% FA (16.8% C16:0; 4.8% C18:0; 21.4% C18:1 *cis*-9; 52.4% C18:2 *cis*-9,*cis*-12; and others <2% each); and (2) Ca salts of palm fatty acids (CSPO; Magnapac, Tectron Inc., Toledo, Parana, Brazil) 85.2% FA (47.8% C16:0; 4.4% C18:0; 37.3% C18:1 *cis*-9; 5.2% C18:2 *cis*-9,*cis*-12; and others <2% each).

³Provided the following per kilogram of product DM: 250 g of Ca, 45 g of P, 65 g of Na, 10 g of Mg, 10 g of S, 2,375 mg of Mn, 2,375 mg of Zn, 562 mg of Cu, 12.5 mg of Co, 31 mg of I, 15.8 mg of Se, 200,000 IU of vitamin A, 50,000 IU of vitamin D₃, 1,250 of vitamin E.

Experimental Measures and Sample Collection During the Treatment Period

Sward height was measured every day at 20 randomized points before animals entered the paddock and after they left. Pre- and postgrazing forage mass were measured in 2 paddocks every week by clipping the forage inside of 4 rectangles (1.5 × 0.75 m) at 3 cm above ground level from sites that represented the mean sward height of the paddock. Total forage mass was weighed and 2 representative subsamples (500 g) taken. The first subsample was dried in a forced-air oven at 55°C for 72 h to determine DM content. The second subsample was separated into leaves, stems, and senescent material. The senescent material was assumed to be any plant part with more than 50% of its superficial area with a yellowish or brownish color.

Forage and concentrate ingredients were collected for chemical composition. Hand-plucked forage samples were taken daily according to De Vries (1995) at 20 randomized points per paddock before cows had access.

The concentrate ingredients were collected once a week. All samples were dried in a forced-air oven at 55°C for 72 h, ground through a 1-mm screen (Wiley mill, Thomas Scientific, Philadelphia, PA), and composited by month.

Forage intake and nutrient digestibility were measured every 4 wk during the treatment period from total fecal excretion and feed indigestibility. To determine fecal excretion, titanium dioxide (TiO₂) was dosed twice a day (20 g/cow per day) after each milking for 15 d. Fecal grab samples were collected after morning and afternoon milkings from the last 5 d, and immediately frozen at -20°C. Subsequently, samples were thawed, dried at 55°C in a forced air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by cow.

Cows were milked twice a day at 0400 and 1500 h, and milk yield was recorded daily. Milk samples from both milking sections were collected once a week and preserved with a bronopol preservative pill. An additional milk sample was collected at each milking every

Table 2. Pasture characteristics at pre- and postgrazing

Characteristic	Pregrazing	SD	Postgrazing	SD
Sward height (cm)	101.4	1.30	62.8	2.21
Forage mass (kg of DM/ha)	10,546	790.5	7,789	475.9
Leaves mass (kg of DM/ha)	4,577	335.2	1,970	147.7
Stem mass (kg of DM/ha)	4,028	323.2	3,489	281.5
Morphological composition (% of forage DM)				
Leaves	43.4	8.21	25.3	7.92
Stem	38.2	6.32	44.8	9.80
Senescent material	18.4	3.51	29.9	14.8
Leaf:stem ratio	1.10	0.10	0.60	0.06

30 d and stored without preservative at -20°C for determination of milk FA profile. Cows were weighed weekly during 2 consecutive days after the morning milking. Body condition was scored weekly by 2 trained investigators on a 5-point scale, where 1 = thin and 5 = fat, as described by Wildman et al. (1982).

Experimental Measures and Sample Collection During the Carryover Period

From 17 to 42 wk postpartum (carryover period), all cows received a common diet fed as TMR once a day at 0900 h. Cows were milked twice a day at 0600 and 1600 h, and milk yield was recorded daily. Milk samples from both milkings were collected once a week and preserved with a bronopol preservative pill. Feed ingredients were collected weekly and stored at -20°C . Subsequently, samples were thawed, dried at 55°C in a forced air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by month. Cows were weighed and BCS recorded weekly as previously described. Dry matter intake was estimated twice during the carryover period from total fecal excretion and feed indigestibility. At wk 24 and 36, fecal excretion was estimated with TiO_2 marker dosed after each milking for 15 d, and fecal samples collected during the last 5 d.

Sample Analysis and Calculations

Composite forage, concentrate, and fecal samples were analyzed for DM by drying samples in an oven at 105°C for 24 h, ash (AOAC International, 2005; method 942.05), total N content by the Dumas combustion method using N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), NDF (Van Soest et al., 1991) with sodium sulfite and heat-stable α -amylase for concentrate samples, ADF and lignin (AOAC International, 2005; method 973.18), and gross energy by bomb calorimeter (Parr Instrument Inc., Moline, IL).

Milk samples were analyzed for fat, protein, lactose, total solids, and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Milk samples for FA profile were extracted according to Hara and Radin (1978) using a 3:2 (vol:vol) mixture of hexane and isopropanol followed by a 67 g/L of sodium sulfate solution. The fat cake was previously obtained by milk centrifugation at $15,000 \times g$ for 30 min at 8°C . Milk FAME were obtained by base-catalyzed transmethylation using a freshly prepared methylation reagent (0.4 mL of 5.4 mol/L of sodium methoxide solution + 1.75 mL of methanol) according to Christie (1982) with modifications (Chouinard et al., 1999). The FAME were determined by GC (model 6890 N; Agilent Technologies Brasil Ltda., Barueri, Brazil) fitted with a flame-ionization detector and equipped with a CP-Sil 88 fused-silica capillary column (100 m $\times$ 0.25 mm $\times$ 0.2 μm film thickness; Varian Inc., Mississauga, ON, Canada). The FAME were identified by comparison with 3 reference FAME standards (Supelco37 mix #47885-U, linoleic acid isomers mix #47791, and CLA isomers mix #05632; Sigma-Aldrich, St. Louis, MO), as described by Kramer et al. (2001) and Cruz-Hernandez et al. (2007). The yield of individual FA in milk fat was calculated by correcting for glycerol content, and other milk lipid classes according to Piantoni et al. (2013).

Forage intake was estimated from total fecal excretion and feed indigestibility. To estimate fecal excretion, fecal samples were analyzed for titanium concentration according to Myers et al. (2004). To determine indigestibility, we determined the indigestible NDF content of forage, concentrate and fecal samples (NDF remaining after 240 h of in situ incubation; Huhtanen et al., 1994). Total fecal excretion, fecal excretion coming from the concentrate, and forage intake were calculated according to de Souza et al. (2015).

Energy values were calculated as described by Harvatin and Allen (2006) as follows: Digestible energy (DE) intake = gross energy (GE) intake $\times$ GE digestibility; NE_L intake was calculated from DE according to NRC (2001). Milk NE_L (Mcal/d) = milk yield (kg)

$\times [0.0929 \times (\text{fat } \%) + 0.0563 \times (\text{true protein } \%) + 0.0395 \times (\text{lactose } \%)]$ (NRC, 2001); NE_L BW gain was calculated according to NRC (2001); and NE_L available for maintenance = NE_L intake – NE_L milk – NE_L BW gain. Energy partitioning was calculated according to Boerman et al. (2015) as percentage allocated to milk, maintenance, or body gain = $[\text{milk energy output, maintenance, or body tissue gain} / (\text{NE}_L \text{ intake}) \times 100]$.

Statistical Analyses

Data were analyzed separately for treatment period (from 3 to 16 wk postpartum) and carryover period (from 17 to 42 wk postpartum). All weekly or monthly data were analyzed using the MIXED procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC) according to the following model as repeated measures:

$$Y_{ijk} = \mu + b_i + C_j + T_k + CT_{jk} + e_{ijk}$$

where Y_{ijk} is the variable of interest, μ is the overall mean, b_i is the random effect of block ($i = 1$ to 12), C_j is the fixed effect of treatment ($j = 1$ to 3), T_k is the fixed effect of time ($k = \text{week or month}$), CT_{jk} is the interaction between treatment and time, and e_{ijk} is the residual error. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Unless otherwise specified, first-order autoregressive was the covariate structure used for analysis because it resulted in the lowest Bayesian information criterion for most of the variables measured. Denominator degrees of freedom were adjusted by the Kenward-Roger method. Means were compared using the Tukey test and differences were declared significant at $P \leq 0.05$, and tendencies were declared at $P \leq 0.10$.

RESULTS

Forage, Concentrate, and Diet Composition

During the treatment period, grazed forage contained (DM basis) 18.3% CP, 54.2% NDF, 3.22% total FA, and 1.76% of C18:3 *cis*-9,*cis*-12,*cis*-15 (Table 1). The control concentrate contained 14.5% CP and 2.71% total FA. The CSSO concentrate contained a greater amount of total FA compared with control (6.60 vs. 2.71% of DM) and mainly increased the amount of C18:2 *cis*-9,*cis*-12 (3.07 vs. 1.24% of DM) in the diet. In contrast, the CSPO concentrate had greater amount of total FA compared with control (6.60 vs. 2.71% of DM), but mainly increased the amount of C16:0 (0.36 vs. 2.22% of DM) in the diet. The carryover diet had,

on a DM basis, 16.6% CP, 37.1% NDF, and 2.0% total FA.

Yields of Milk and Milk Components

During the treatment period, CSPO increased milk yield compared with control and CSSO ($P < 0.01$; Table 3). Also, CSSO increased milk yield compared with control ($P < 0.01$). Compared with control, CSPO reduced milk protein content ($P < 0.01$) but did not affect contents of milk fat or milk lactose. In contrast, CSSO reduced milk fat content ($P < 0.01$) and milk protein content ($P < 0.01$) compared with control. The CSPO treatment increased yields of milk fat, milk protein, and milk lactose compared with CSSO and control ($P < 0.01$). The CSSO treatment increased lactose yield ($P = 0.01$) but decreased milk fat yield compared with control ($P < 0.01$). As a result, we observed no differences in 3.5% FCM ($P = 0.98$) or ECM ($P = 0.95$) between CSSO and control. The CSPO treatment increased 3.5% FCM, ECM, and cumulative milk yield compared with control and CSSO ($P < 0.01$). The CSSO treatment also increased cumulative milk yield in compared with control ($P < 0.01$). Both CSPO and CSSO increased feed efficiency (3.5% FCM/DMI) compared with control ($P < 0.01$), and CSPO also increased feed efficiency compared with CSSO ($P < 0.01$).

During the carryover period, no differences in milk composition were observed among the treatments ($P > 0.10$, Table 4). A treatment $\times$ time interaction was observed during the carryover period for milk yield ($P = 0.01$; Figure 1). This was due to CSPO maintaining higher milk production compared with control and CSSO until 30 wk postpartum ($P < 0.05$), whereas CSSO maintained higher milk yield compared with control ($P < 0.05$) until 25 wk postpartum. The CSPO treatment increased milk yield and yield of milk components compared with control and CSSO ($P < 0.05$), whereas CSSO only tended to increase these variables compared with control ($P = 0.10$). Furthermore, CSPO increased 3.5% FCM, ECM, and cumulative milk yield compared with control and CSSO ($P < 0.01$), whereas CSSO increased these variables compared with control ($P = 0.05$).

Intake and Total-Tract Digestibility

During the treatment period, we observed no interaction between treatments and time for all variables related to intake and digestibility ($P > 0.10$; Table 5). The CSSO treatment reduced DMI compared with control ($P = 0.05$) and CSPO ($P = 0.03$), which represented a decrease in forage intake (as the amount of concentrate fed daily was the same among treat-

Table 3. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on production, milk composition and feed efficiency of dairy cows during the treatment period (from wk 3 to 16)

Item	Treatment (Trt)			SEM	P-value ¹		
	Control	CSSO	CSPO		Trt	Time	Trt × time
Yield (kg/d)							
Milk	24.2 ^c	26.8 ^b	29.0 ^a	0.52	<0.01	<0.01	0.21
Fat	0.86 ^b	0.78 ^c	0.95 ^a	0.02	<0.01	<0.01	0.18
Protein	0.80 ^b	0.83 ^b	0.89 ^a	0.02	<0.01	<0.01	0.16
Lactose	1.15 ^c	1.21 ^b	1.36 ^a	0.02	<0.01	<0.01	0.19
3.5% FCM ²	24.1 ^b	24.1 ^b	27.9 ^a	0.49	<0.01	<0.01	0.23
ECM ³	24.8 ^b	24.8 ^b	28.2 ^a	0.54	<0.01	<0.01	0.24
Cumulative milk yield (kg)	2,160 ^c	2,376 ^b	2,565 ^a	44.2	<0.01	NA ⁴	NA
Feed efficiency ⁵	1.39 ^c	1.44 ^b	1.57 ^a	0.03	<0.01	<0.01	0.45
Milk composition (%)							
Fat	3.50 ^a	2.89 ^b	3.39 ^a	0.08	<0.01	<0.01	0.25
Protein	3.31 ^a	3.10 ^b	3.10 ^b	0.05	<0.01	<0.01	0.29
Lactose ⁶	4.60	4.52	4.60	0.06	0.78	<0.01	0.34
MUN (mg/dL)	11.2	10.6	11.9	0.10	0.20	0.34	0.78
BW (kg)	475	487	476	9.87	0.08	<0.01	0.02
BW change ⁷ (kg)	-2.03	-0.73	-8.54	3.34	0.05	<0.01	0.05
BCS	2.84	2.88	2.70	0.13	0.11	<0.01	0.04
BCS change ⁷	-0.03	-0.01	-0.12	0.04	0.05	<0.01	0.03

^{a-c}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$). Separation was only conducted if treatment effect was $P \leq 0.05$.

¹Probability associated with the effects of treatment, time, and treatment × time interaction.

²3.5% FCM = [(0.4324 × milk yield) + (16.216 × fat yield)].

³ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

⁴Not applicable.

⁵Feed efficiency = 3.5% FCM/DMI.

⁶Because of infinite likelihood with first-order autoregressive, the variance-covariance structure used to analyze was unstructured.

⁷Calculated as the difference in BW or BCS every 4 wk.

Table 4. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on DMI and production responses of dairy cows during the carryover period (from wk 17 to 42)

Item	Treatment (Trt)			SEM	P-value ¹		
	Control	CSSO	CSPO		Trt	Time	Trt × time
DMI (kg/d)	19.6	19.6	19.9	0.45	0.28	0.25	0.44
Yield (kg/d)							
Milk	18.4 ^b	19.3 ^b	21.0 ^a	0.44	<0.01	<0.01	0.01
Fat	0.77 ^b	0.81 ^b	0.88 ^a	0.03	<0.01	<0.01	0.09
Protein	0.68 ^b	0.69 ^b	0.75 ^a	0.03	<0.01	<0.01	0.12
Lactose	0.81 ^b	0.85 ^b	0.92 ^a	0.04	<0.01	<0.01	0.06
3.5% FCM ²	20.5 ^c	21.6 ^b	23.4 ^a	0.48	<0.01	<0.01	0.07
ECM ³	20.9 ^c	21.8 ^b	23.7 ^a	0.47	<0.01	<0.01	0.07
Cumulative milk yield (kg)	3,349 ^c	3,513 ^b	3,822 ^a	55.7	<0.01	NA ⁴	NA
Milk composition (%)							
Fat	4.20	4.25	4.21	0.07	0.24	<0.01	0.18
Protein	3.62	3.59	3.59	0.05	0.45	<0.01	0.35
Lactose	4.41	4.42	4.40	0.04	0.69	<0.01	0.24
MUN (mg/dL)	10.2	10.8	10.6	0.14	0.34	0.65	0.76
BW (kg)	518	532	515	15.5	0.12	<0.01	0.04
BW change ⁵ (kg)	8.66	8.41	11.5	5.11	0.18	<0.01	0.07
BCS	3.27	3.30	3.18	0.14	0.15	<0.01	0.05
BCS change ⁵	0.10	0.10	0.13	0.01	0.10	<0.01	0.09

^{a-c}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$). Separation only conducted if treatment effect was $P \leq 0.05$.

¹Probability associated with the effects of treatment, time, and treatment × time interaction.

²3.5% FCM = [(0.4324 × milk yield) + (16.216 × fat yield)].

³ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

⁴Not applicable.

⁵Calculated as the difference in BW or BCS every 4 wk.

ments). Because the diets had similar concentrations of nutrients, the intakes of OM ($P = 0.04$) and NDF ($P = 0.05$) were also decreased by CSSO compared with control. Both fat treatments increased FA intake compared with control ($P < 0.01$). The CSPO treatment tended to increase CP intake compared with CSSO ($P = 0.07$). Treatments did not affect CP digestibility ($P = 0.15$). Both fat treatments decreased FA digestibility compared with control ($P < 0.01$), and CSPO increased NDF digestibility compared with control and CSSO ($P = 0.03$). As a result, CSPO increased digestibilities of DM ($P = 0.05$) and OM ($P = 0.04$) compared with control and CSSO. Although CSSO reduced NDF digestibility compared with control ($P = 0.04$), no differences in DM and OM digestibilities were observed between these treatments ($P = 0.54$). During the carryover period, no difference in DMI was observed among the treatments ($P = 0.28$, Table 4).

BW and BCS

Treatment $\times$ time interactions during the treatment period (Table 3) were observed for BW ($P = 0.02$), change in BW ($P = 0.05$), BCS ($P = 0.04$), and change

in BCS ($P = 0.03$). At wk 7 postpartum, no difference between treatments was detected for BW ($P = 0.21$; Figure 2) or BCS ($P = 0.19$; Figure 3). In contrast, at wk 12 and 16 postpartum, CSSO increased BW compared with control and CSPO ($P = 0.05$). In addition, CSSO tended to decrease BW ($P = 0.10$) and BCS ($P = 0.07$) loss during wk 3 to 7 postpartum compared with control and CSPO. Also, CSSO during wk 8 to 12 postpartum decreased BW ($P = 0.05$) and BCS ($P = 0.05$) loss compared with control and CSPO. Overall during the treatment period, CSSO tended to increase BW ($P = 0.08$) and reduced BW and BCS changes ($P = 0.05$) compared with the other treatments.

During the carryover period, a treatment $\times$ time interaction was observed for BCS ($P = 0.05$; Table 4) and BW ($P = 0.04$), and tendencies for BW change ($P = 0.07$) and BCS change ($P = 0.09$). This occurred because CSSO increased BW (Figure 2) and BCS (Figure 3) compared with the other treatments during wk 20 to 28 postpartum ($P < 0.05$). After 28 wk postpartum, no differences in BW and BCS were detected among treatments ($P > 0.10$). Also, CSPO tended to increase BW change and BCS change during wk 28 to 36 ($P < 0.10$) compared with the other treatments.

Figure 1. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on milk yield (kg/d) over time during the treatment and carryover periods. * P -values were significant ($P < 0.05$) for treatment effects. Error bars represent SEM.

Figure 2. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on BW and BW change over time during the treatment and carryover periods. **P*-values were significant ($P < 0.05$) for treatment effects. Error bars represent SEM.

Figure 3. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on BCS and BCS change over time during the treatment and carryover periods. The line on the wk 16 indicates the start of the carryover period. **P*-values were significant ($P < 0.05$) for treatment effects. Error bars represent SEM.

Table 5. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on nutrients intake, and apparent total-tract digestibility of dairy cows during the treatment period (from wk 3 to 16)

Item	Treatment (Trt)			SEM	<i>P</i> -value ¹		
	Control	CSSO	CSPO		Trt	Time	Trt × time
Intake (kg/d)							
DM	17.9 ^a	17.2 ^b	18.0 ^a	0.42	0.04	<0.01	0.45
OM	16.4 ^a	15.5 ^b	16.7 ^a	0.38	0.04	<0.01	0.47
CP	3.0	2.92	3.10	0.08	0.07	0.02	0.58
NDF	6.2 ^a	5.7 ^b	6.5 ^a	0.23	0.05	<0.01	0.47
Fatty acids	0.40 ^b	0.69 ^a	0.71 ^a	0.04	<0.01	0.02	0.50
Digestibility (%)							
DM	62.3 ^b	63.3 ^b	66.5 ^a	1.35	0.05	0.61	0.66
OM	64.2 ^b	65.2 ^b	68.2 ^a	1.28	0.04	0.62	0.87
CP	64.4	65.5	65.8	1.21	0.15	0.44	0.67
NDF	52.4 ^b	47.7 ^c	56.5 ^a	1.93	0.03	0.19	0.58
Fatty acids	85.2 ^a	81.5 ^b	80.8 ^b	1.66	<0.01	0.27	0.52

^{a-c}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$). Separation was only conducted if treatment effect was $P \leq 0.05$.

¹Probability associated with the effects of treatment, time, and treatment × time interaction.

Energy Calculations and Energy Partitioning

No treatment $\times$ time interactions were observed for variables related to energy partitioning (Table 6). The CSPO treatment increased NE_L intake compared with control ($P = 0.01$) and CSSO ($P = 0.03$). Also, CSPO increased NE_L in milk ($P < 0.01$) and NE_L lost as BW gain ($P = 0.05$) compared with the other treatments. No differences among treatments were detected for energy available for maintenance ($P = 0.48$). When considering energy partitioning (as % energy intake), CSPO increased energy partitioning toward milk ($P = 0.01$) and increased energy mobilized from body reserves compared with control and CSSO ($P = 0.01$). Also, CSSO tended to reduce the mobilization of energy from body reserves compared with control ($P = 0.09$). No differences among treatments were detected for energy partitioned to maintenance ($P = 0.67$).

Milk FA Yields and Profile

No treatment $\times$ time interactions were observed for milk FA yield or profile ($P > 0.10$; Table 7). Both CSPO and CSSO treatments decreased the concentration of de novo FA compared with control ($P < 0.01$). In contrast, CSPO increased the concentration of mixed FA ($P = 0.04$) compared with the other treatments. Also, CSSO increased the concentration of preformed FA ($P = 0.04$) compared with the other treatments. The CSSO treatment reduced the concentration of SFA ($P = 0.01$), and increased the concentration of MUFA ($P = 0.04$) and PUFA ($P = 0.02$). Additionally, CSSO increased milk

C18:1 *trans*-10 and C18:2 *trans*-10,*cis*-12 concentrations compared with the other treatments ($P < 0.01$; Supplementary Table S1; <https://doi.org/10.3168/jds.2016-11636>).

For yields of milk FA, CSPO decreased the yield of de novo FA compared with control ($P < 0.01$), whereas CSSO decreased the yield of de novo FA compared with control and CSPO ($P < 0.01$). The CSPO treatment increased the yield of mixed FA ($P < 0.01$) compared with the other treatments, which was mainly a result of the increase in yield of C16:0. In contrast, CSSO increased the yield of preformed FA ($P = 0.04$) compared with other treatments due to the increase in the yield of most 18-carbon milk FA. Additionally, CSSO reduced yield of milk SFA ($P < 0.01$) but increased the yields of milk MUFA ($P < 0.01$) and PUFA ($P < 0.01$).

DISCUSSION

Pasture-based systems including temperate and tropical grasses and legumes are generally the most cost-effective sources of nutrients to feed dairy cows (Peyraud and Delaby, 2001). However, some of the challenges of these systems include the seasonal variation in pasture availability and nutritive value (Roche et al., 2009a) and the ability to meet nutritional requirements of high-producing cows (Peyraud and Delagarde, 2013). The challenge of meeting nutritional requirements is greater during early lactation when cows generally enter a period of negative energy balance (NRC, 2001). To overcome these limitations, we evaluated whether feeding different sources of supplemental fat during

Table 6. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on energy balance and energy partitioning of dairy cows during the treatment period (from wk 3 to 16)

Item	Treatment (Trt)				P-value ¹		
	Control	CSSO	CSPO	SEM	Trt	Time	Trt $\times$ time
NE_L intake ² (Mcal/d)	27.6 ^b	28.0 ^b	29.2 ^a	0.62	0.01	0.01	0.46
Production							
Milk NE_L ³ (Mcal/d)	16.8 ^b	16.4 ^b	19.3 ^a	0.37	<0.01	<0.01	0.76
NE_L BW change ⁴ (Mcal/d)	-0.36 ^b	-0.13 ^b	-1.52 ^a	0.04	0.05	<0.01	0.10
NE_L available maint ⁵ (Mcal/d)	11.2	11.7	11.4	0.65	0.48	<0.01	0.18
Energy partitioning (%)							
Milk	60.9 ^b	58.6 ^b	66.1 ^a	1.15	0.01	<0.01	0.45
Reserves	-1.31 ^b	-0.52 ^b	-5.23 ^a	0.22	0.01	<0.01	0.12
Maintenance	40.4	41.9	39.1	0.85	0.67	<0.01	0.23

^{a,b}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$). Separation was only conducted if treatment effect was $P \leq 0.05$.

¹Probability associated with the effects of treatment, time, and treatment $\times$ time interaction.

² $NE_{L(intake)} = DMI \times NE_{L(diet)}$.

³ $NE_{L(milk)} = \text{Milk yield (kg)} \times (0.0929 \times \text{fat \%} + 0.0563 \times \text{true protein \%} + 0.0395 \times \text{lactose \%})$ (NRC, 2001).

⁴ $NE_{L(BW change)}$ calculated according to NRC (2001).

⁵ NE_L available for maintenance = $NE_{L(intake)} - NE_{L(BW gain)} - NE_{L(milk)}$.

Table 7. Effects of supplementation with calcium salts of soybean (CSSO) or palm fatty acids (CSPO) on summation of milk fatty acids profile and yield of dairy cows during the treatment period (from wk 3 to 16)

Item	Treatment (Trt)			SEM	P-value ¹		
	Control	CSSO	CSPO		Trt	Time	Trt × time
Profile (g/100 g of FA)							
Summation by source ²							
Σ <C16	25.6 ^a	20.8 ^b	21.4 ^b	0.99	<0.01	0.97	0.31
Σ C16 + C16:1	30.7 ^b	30.2 ^b	33.9 ^a	1.40	0.04	0.29	0.91
Σ >C16	43.7 ^b	49.0 ^a	44.7 ^b	2.20	0.04	0.30	0.31
Summation by saturation							
Σ SFA	66.9 ^a	61.5 ^b	64.1 ^a	1.76	0.01	0.98	0.49
Σ MUFA	28.9 ^b	33.6 ^a	31.6 ^{ab}	1.30	0.04	0.68	0.55
Σ PUFA	4.21 ^b	4.92 ^a	4.33 ^b	0.24	0.02	0.18	0.84
Yield (g/d)							
Summation by source							
Σ <C16	203 ^a	149 ^c	187 ^b	20.3	<0.01	<0.01	0.65
Σ C16 + C16:1	243 ^b	217 ^b	296 ^a	24.3	<0.01	<0.01	0.56
Σ >C16	346 ^b	352 ^b	391 ^a	34.6	0.04	<0.01	0.48
Summation by saturation							
Σ SFA	529 ^a	441 ^b	560 ^a	37.1	<0.01	<0.01	0.67
Σ MUFA	229 ^b	241 ^b	276 ^a	16.0	<0.01	<0.01	0.76
Σ PUFA	31.2 ^b	35.2 ^a	37.6 ^a	2.30	<0.01	<0.01	0.84

^{a-c}Means within a row with different superscripts differ by the Tukey test ($P \leq 0.05$). Separation was only conducted if treatment effect was $P \leq 0.05$.

¹Probability associated with the effects of treatment, time, and treatment × time interaction.

²De novo FA originate from mammary de novo synthesis (<16 carbons), preformed FA originate 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 FA are reported in Supplementary Tables S1 and S2, respectively (<https://doi.org/10.3168/jds.2016-11636>).

early lactation to high-producing grazing dairy cows would increase energy intake and performance, and whether supplementation during early lactation would have carryover effects throughout lactation.

Effects of fat supplementation on milk production have been inconsistent across studies, which is likely associated with the fat source used (Rabiee et al., 2012). In our study, both fat supplements increased milk yield by 15% compared with control during the treatment period. This could be due to the greater energy density of the fat-supplemented diets providing more energy for metabolic functions. As discussed by Palmquist (1994), fat supplementation increases energetic efficiency in lactating cows by increasing total energy intake, by generating ATP more efficiently compared with VFA, and by directly incorporating long-chain FA into milk fat. Also, one mechanism proposed for the increase in milk yield with fat supplementation is glucose sparing, in which the suppression of de novo FA synthesis in the mammary gland may decrease the oxidative use of glucose to generate reducing equivalents for milk fat synthesis (Storry et al., 1973). This extra glucose may be utilized in other milk synthetic processes including lactose synthesis and increased milk yield (Palmquist and Jenkins 1980). In our study, both fat supplements decreased de novo milk FA synthesis, so that the aforementioned mechanism may be at least in part

responsible for these results. Schroeder et al. (2004) compiled 18 studies feeding different fat supplements to cows grazing on temperate pastures and observed that both saturated and unsaturated fat supplements increased milk yield by, on average, 5%. The greater response in our study to supplemental fat compared with Schroeder et al. (2004) review may be associated with the greater production level of our cows. Furthermore, it is possible that high-producing dairy cows grazing on tropical pastures had lower energy intake due to the lower nutritive value and forage intake of tropical pastures compared with temperate pastures (Da Silva et al., 2013), which would suggest that they are more responsive to fat supplementation. This hypothesis deserves further research.

The CSPO treatment increased not only milk yield but also yields of milk components compared with CSSO and control, resulting in greater energy output in milk. The possible explanation for the better performance during the treatment period of cows receiving CSPO compared with CSSO was their distinct effects on forage intake and nutrient digestibility; CSPO increased NDF digestibility compared with other treatments. Similarly, Weld and Armentano (2015), in a recent meta-analysis, observed that supplementation with calcium salts of palm FA increased NDF digestibility by 2.2 percentage units. Also, in our study, both fat supplements resulted

in a minor decrease in total FA digestibility compared with control. The greater intake and flow of FA is associated with the reduction in FA digestibility when fat supplements are fed (Boerman et al., 2015). Therefore, the positive effects of CSPO supplementation on NDF digestibility with no negative effect on forage intake and minor effects on FA digestibility resulted in greater NE_L intake for this treatment and better milk production responses during the treatment period.

We observed deleterious effects of CSSO supplementation on forage intake, leading to lower total DMI. Although the energy contribution of concentrate was greater for CSSO compared with control, because of the reduction in forage intake, these treatments had similar NE_L intakes. The effect of fat supplements on DMI is variable and usually depends on the type of fat being fed (Rabiee et al., 2012). For dairy cows fed TMR, studies have shown that the hypophagic effect of feeding fat seems to be more pronounced for unsaturated than for saturated fat supplements (Harvatine and Allen, 2006; Relling and Reynolds, 2007), with DMI decreasing linearly as the degree of unsaturation increases (Pantoja et al., 1994). The more pronounced decrease in DMI for unsaturated FA is likely mediated at least in part by increased secretion of cholecystokinin (Bradford et al., 2008). He et al. (2012) observed that DMI was linearly reduced as the C18:2 *cis*-9,*cis*-12 concentration increased in the diet (from 1.51 to 3.86% of diet DM). In grazing cows, Schroeder et al. (2004) observed that forage intake and total DMI were not affected by fat supplementation; however, it is important to note that, in that review, only 6 studies measured forage intake mostly with saturated fat supplements. Altogether, our results demonstrate that the degree of saturation of fat supplements affects forage intake similarly to the results observed in cows fed TMR.

Supplementation with CSSO reduced milk fat content and milk fat yield, resulting in no increase in FCM or energy output in milk compared with control. Although calcium salts protection can reduce the deleterious effects of feeding fat to ruminal population, its stability is largely dependent on rumen pH and the degree of unsaturation of the fat source (Sukhija and Palmquist, 1990). Milk fat depression (MFD) is classically observed in cows fed diets that contain plant or fish oil supplements (Bauman and Griinari, 2001). The biohydrogenation theory proposes that under specific dietary conditions, intermediates resulting from altered ruminal biohydrogenation act on the mammary gland inhibiting fat synthesis (Bauman et al., 2011). Changes in milk fat yield are negatively correlated with milk fat concentration of some *trans* FA (Harvatine et al., 2009b). The CLA *trans*-10,*cis*-12 produced as inter-

mediates in the rumen biohydrogenation process have been shown to reduce milk fat concentration and yield (Bauman et al., 2011). In our study, we observed that CSSO supplementation increased several milk *trans* FA, including CLA *trans*-10,*cis*-12 in milk fat. Conjugated linoleic acid *trans*-10,*cis*-12 downregulates enzymes involved in de novo milk FA synthesis (i.e., acetyl-CoA carboxylase, fatty acid synthase) in the mammary gland (Harvatine and Bauman, 2006). Therefore, the decrease in milk fat yield of CSSO-supplemented cows in the present study was likely accomplished by greater dissociation of calcium salts, resulting in a greater pool of some *trans* FA that acted on the mammary gland inhibiting milk FA synthesis.

Milk fat represents about 50% of milk energy output, and it is the major reason for the high energy demands resulting in mobilization during early lactation, mainly in the form of body fat (von Soosten et al., 2012). It is well established that during MFD there is a decreased priority for milk production and an increased priority for storage of energy as adipose (Van Soest, 1963; Griinari and Bauman, 2006). This likely explains why the CSSO treatment reduced BW and BCS loss during the treatment period. Boerman et al. (2015) observed a positive correlation between *trans*-10 C18:1 concentration in milk fat and change in BCS. Typically, the concentration of C18:1 *trans*-10 is elevated in milk fat during MFD, and we observed a greater concentration of this FA in the CSSO treatment. Harvatine et al. (2009a) reported that during abomasal infusions of CLA *trans*-10,*cis*-12, lipogenic genes in mammary tissue were downregulated, whereas expression of lipogenic enzymes in adipose tissue underwent a significant increase. Therefore, diets that cause MFD result in decreased FCM yield but might repartition more energy toward body reserves, as we observed in our study. Hence, diet-induced MFD is detrimental in the short term because of decreased milk income but may have positive effects in early lactation due to increases in energy balance and possibly improvements in fertility (de Veth et al., 2009).

One of our objectives was to evaluate the potential carryover effect of fat supplementation to early lactation cows on production responses throughout lactation. The carryover effect may be important in determining the economic impact of specific feeding strategies because fat supplementation is generally expensive compared with other dietary components or ingredients. Usually, each extra kilogram of milk secreted at peak production results in approximately 200 kg of milk subsequent during lactation (Roche et al., 2013), suggesting that supplementation during early lactation that increases milk production at peak could have positive effects even

after supplementation ceases. In our study, we observed that both fat supplements had a positive carryover effect on milk yield, but CSPO was able to maintain higher production compared with control and CSSO until 30 wk postpartum, whereas CSSO had a lower carryover effect, sustaining higher milk yield compared with control until 25 wk postpartum. These results agree with a recent review in which most results showed that a more generous feeding in early lactation caused a positive carryover effect on subsequent production (Jørgensen et al., 2016). The possible reasons for this carryover effect involve an increase in either mammary cell number (Akers, 2002) or cell secretory activity (Nørgaard et al., 2005). Also, as discussed by Roche et al. (2013), an increase in DMI could occur in cows that were previously supplemented because of increased hunger associated with their greater milk production when the supplements were removed. This explanation likely does not apply in our study, because we did not observe differences in DMI during the carryover period. Further studies are needed to comprehend factors associated with carryover effects and to determine the duration and magnitude of this under different dietary conditions.

Although CSPO supplementation increased cumulative milk production, this treatment resulted in greater BCS loss and greater BCS variation throughout lactation. Similar to our results, Roche et al. (2007) reported that greater postcalving BCS loss increased the height of the lactation profile and the rate of milk yield increase to peak, but reduced lactation persistency. The association between the general shape of the lactation curve and calving BCS, and also BCS change between calving and nadir, are nonlinear (Roche et al., 2009b). Roche et al. (2007) reported such nonlinear associations, with lactation milk yield increasing with BCS loss after calving, up to 0.5 BCS units, and decreasing with further BCS loss. These findings corroborated our results, where BCS loss from CSPO supplementation was 0.4 BCS units. During the carryover period, BCS and BW were lower for CSPO-supplemented cows up to 32 wk postpartum. After 32 wk postpartum, these cows recovered more body condition simultaneously with the reduction in milk production. In contrast, we observed that CSSO not only reduced BW loss during the treatment period, but also increased BW and BCS compared with other treatments during the carryover period (wk 20 to 28 postpartum). Although CSSO had a lesser carryover effect on milk production compared with CSPO, the lower variation in BCS, especially at the beginning of lactation, indicates a reduction in negative energy balance. The association between diets that improve energy balance and reproductive performance deserves further research.

CONCLUSIONS

Supplementation with calcium salts of FA to early lactation dairy cows grazing tropical pasture altered energy partitioning and had a positive carryover effect on milk production. Although supplementation with calcium salts of palm FA increased milk production and yield of milk components, supplementation with calcium salts of soybean FA reduced DMI and milk fat yield but resulted in lower mobilization of reserves and less BW and BCS variation throughout lactation.

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REFERENCES

- Akers, R. M. 2002. Lactation and the Mammary Gland. Iowa State University Press, Ames.
- AOAC International. 2005. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.
- Auldist, M. J., L. C. Marett, J. S. Greenwood, M. Hannah, J. L. Jacobs, and W. J. Wales. 2013. Effects of different strategies for feeding supplements on milk production responses in cows grazing a restricted pasture allowance. *J. Dairy Sci.* 96:1218–1231.
- Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. *J. Dairy Sci.* 86:1–42.
- Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. *Livest. Prod. Sci.* 70:15–29.
- Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. *Annu. Rev. Nutr.* 31:299–319.
- Boerman, J. P., S. B. Potts, M. J. VandeHaar, and A. L. Lock. 2015. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. *J. Dairy Sci.* 98:7264–7276.
- Bradford, B. J., K. J. Harvatine, and M. S. Allen. 2008. Dietary unsaturated fatty acids increased plasma glucagon-like peptide-1 and cholecystokinin and may decrease premeal ghrelin in lactating dairy cows. *J. Dairy Sci.* 91:1443–1450.
- Chouinard, P. Y., L. Corneau, A. Sæbø, and D. E. Bauman. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. *J. Dairy Sci.* 82:2737–2745.
- Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. *J. Lipid Res.* 23:1072–1075.
- Cruz-Hernandez, C., J. K. G. Kramer, J. J. Kennelly, D. R. Glimm, B. M. Sorensen, E. K. Okine, L. A. Goonewardene, and R. J. Weselake. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *J. Dairy Sci.* 90:3786–3801.
- Da Silva, S. C., F. M. A. Gimenes, D. O. L. Sarmento, A. F. Sbrissia, D. E. Oliveira, A. Hernandez-Garay, and A. V. Pires. 2013. Grazing behaviour, herbage intake and animal performance of beef cattle

- heifers on Marandu palisadegrass subjected to intensities of continuous stocking management. *J. Agric. Sci.* 151:727–739.
- de Souza, J., F. Batistel, K. C. Welter, M. M. V. Silva, D. F. Costa, and F. A. P. Santos. 2015. Evaluation of external markers to estimate fecal excretion, intake and digestibility in dairy cows. *Trop. Anim. Health Prod.* 47:265–268.
- de Veth, M. J., D. E. Bauman, W. Koch, G. E. Mann, A. M. Pfeiffer, and W. R. Butler. 2009. Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows. *J. Dairy Sci.* 92:2662–2669.
- De Vries, M. F. W. 1995. Estimating forage intake and quality in grazing cattle: A reconsideration of the hand-plucking method. *J. Range Manage.* 48:370–375.
- Dohme-Meier, F., L. D. Kaufmann, S. Görs, P. Junghans, C. C. Metzges, H. A. van Dorland, R. M. Bruckmaier, and A. Mürger. 2014. Comparison of energy expenditure, eating pattern and physical activity of grazing and zero-grazing dairy cows at different time points during lactation. *Livest. Sci.* 162:86–96.
- Doyle, P. T., S. A. Francis, and C. R. Stockdale. 2005. Associative effects between feeds when concentrate supplements are fed to grazing dairy cows: A review of likely impacts on metabolisable energy supply. *Aust. J. Agric. Res.* 56:1315–1329.
- Fonseca, L., P. C. F. Carvalho, J. C. Mezzalana, C. Bremm, J. R. Galli, and P. Gregorini. 2013. Effect of sward surface height and level of herbage depletion on bite features of cattle Grazing *Sorghum bicolor* swards. *J. Anim. Sci.* 91:4357–4365.
- Grinari, J. M., and D. E. Bauman. 2006. Milk fat depression: concepts, mechanisms and management applications. Pages 389–409 in *Ruminant Physiology Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*. Wageningen Academic Publ., Wageningen, the Netherlands.
- Hara, A., and N. S. Radin. 1978. Lipid extraction with low-toxicity solvent. *Anal. Biochem.* 90:420–426.
- Harvatine, 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.
- Harvatine, K. J., and D. E. Bauman. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. *J. Nutr.* 136:2468–2474.
- Harvatine, K. J., Y. R. Boisclair, and D. E. Bauman. 2009b. Recent advances in the regulation of milk fat synthesis. *Animal* 3:40–54.
- Harvatine, K. J., J. W. Perfield, and D. E. Bauman. 2009a. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. *J. Nutr.* 139:849–854.
- He, M., K. L. Perfield, H. B. Green, and L. E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. *J. Dairy Sci.* 95:1447–1461.
- Hills, J. L., W. J. Wales, F. R. Dunshea, S. C. Garcia, and J. R. Roche. 2015. Invited review: An evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. *J. Dairy Sci.* 98:1363–1401.
- Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Anim. Feed Sci. Technol.* 48:211–227.
- Jørgensen, C. H., R. Spörndly, J. Bertilsson, and S. Østergaard. 2016. Invited review: Carryover effects of early lactation feeding on total lactation performance in dairy cows. *J. Dairy Sci.* 99:3241–3249.
- Kramer, J. K., C. Cruz-Hernandez, and J. Q. Zhou. 2001. Conjugated linoleic acids and octadecenoic acids: Analysis by GC. *Eur. J. Lipid Sci. Technol.* 103:600–609.
- Lock, A. L., and J. de Souza. 2016. Fatty acid digestibility and impacts on responses of dairy cows. Pages 78–89 in *Proc. 27th Annu. Florida Rumin. Nutr. Symp. Univ. Florida, Gainesville*.
- Muller, L. D., and S. L. Fales. 1998. Supplementation of cool-season grass pastures for dairy cattle. Pages 335–350 in *Grass for Dairy Cattle*. J. H. Cherney and D. J. R. Cherney, ed. CAB International, Wallingford, UK.
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183.
- Nørgaard, J., A. Sørensen, M. T. Sørensen, J. B. Andersen, and K. Sejrsen. 2005. Mammary cell turnover and enzyme activity in dairy cows: Effects of milking frequency and diet energy density. *J. Dairy Sci.* 88:975–982.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Palmquist, D. L. 1994. The role of dietary fats in efficiency of ruminants. *J. Nutr.* 124:1377S–1382S.
- Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations. *J. Dairy Sci.* 63:1–14. (review).
- Pantoja, J., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production. *J. Dairy Sci.* 77:2341–2356.
- Peyraud, J. L., and L. Delaby. 2001. Ideal concentrate feeds for grazing dairy cows—Responses to supplementation in interaction with grazing management and grass quality. Page 203 in *Recent Advances in Animal Nutrition*. P. C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.
- Peyraud, J. L., and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply under grazing. *Animal* 7:57–67.
- 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.
- Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. *J. Dairy Sci.* 95:3225–3247.
- Relling, A. E., and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and plasma concentrations of gut peptides in lactating dairy cows. *J. Dairy Sci.* 90:1506–1515.
- Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009b. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *J. Dairy Sci.* 92:5769–5801.
- Roche, J. R., J. K. Kay, A. G. Rius, T. M. Grala, A. J. Sheahan, H. M. White, and C. V. C. Phyn. 2013. Short communication: Immediate and deferred milk production responses to concentrate supplements in cows grazing fresh pasture. *J. Dairy Sci.* 96:2544–2550.
- Roche, J. R., J. M. Lee, K. A. Macdonald, and D. P. Berry. 2007. Relationships among body condition score, body weight, and milk production variables in pasture-based dairy cows. *J. Dairy Sci.* 90:3802–3815.
- Roche, J. R., L. R. Turner, J. M. Lee, D. C. Edmeades, D. J. Donaghy, K. A. Macdonald, J. W. Penno, and D. P. Berry. 2009a. Weather, herbage quality and milk production in pastoral systems. 2. Temporal patterns and intra-relationships in herbage quality and mineral concentration parameters. *Anim. Prod. Sci.* 49:200–210.
- Santos, F. A. P., J. R. R. Dórea, J. de Souza, F. Batistel, and D. F. A. Costa. 2014. Forage management and methods to improve nutrient intake in grazing cattle. Pages 144–164 in *Proc. 25th Annu. Florida Rumin. Nutr. Symp. Univ. Florida, Gainesville*.
- Schroeder, G. F., G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller. 2004. Effects of fat supplementation on milk production and composition by dairy cows on pasture: A review. *Livest. Prod. Sci.* 86:1–18.
- Storry, J. E., A. J. Hail, and V. W. Johnson. 1973. The effects of increasing amounts of dietary tallow on milk-fat secretion in the cow. *J. Dairy Res.* 40:293–299.
- Sukhija, P. S., and D. L. Palmquist. 1990. Dissociation of Ca soaps of long-chain fatty acids in rumen fluid. *J. Dairy Sci.* 73:1784–1787.
- Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency: A review. *J. Dairy Sci.* 46:204–216.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.

- Vilela, D., M. J. Alvim, L. L. Matos, and J. B. Matioli. 2002. Use of rumen bypass fat for early-lactating dairy cows grazing on coast-cross pasture. *Pesqui. Agropecu. Bras.* 37:1503–1509.
- Voltolini, T. V., F. A. P. Santos, J. C. Martinez, H. Imaizumi, R. L. Clarindo, and M. A. Penati. 2010. Milk production and composition of dairy cows grazing elephant grass under two grazing intervals. *Braz. J. Anim. Sci.* 39:121–127.
- von Soosten, D., U. Meyer, M. Piechotta, G. Flachowsky, and S. Dänicke. 2012. Effect of conjugated linoleic acid supplementation on body composition, body fat mobilization, protein accretion, and energy utilization in early lactation dairy cows. *J. Dairy Sci.* 95:1222–1239.
- Wales, W. J., and P. T. Doyle. 2003. Effect of grain and straw supplementation on marginal milk production responses and rumen function of cows grazing highly digestible subterranean clover pasture. *Aust. J. Exp. Agric.* 43:467–474.
- Wales, W. J., E. S. Kolver, P. L. Thorne, and A. R. Egan. 2004. Diurnal variation in ruminal pH on the digestibility of highly digestible perennial ryegrass during continuous culture fermentation. *J. Dairy Sci.* 87:1864–1871.
- 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.
- Weld, K. A., and L. E. Armentano. 2015. Supplementing lactating cow diets with long chain fats has minimal effects on total-tract NDF digestibility: A quantitative review. *J. Dairy Sci.* 98(E-Suppl. 1):452. (Abstr.)
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.