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Increasing levels of calcium salts of palm fatty acids affect production responses during the immediate postpartum and carryover periods in dairy cows

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ABSTRACT

Our objective was to determine the dose-response effects of calcium salts of palm fatty acids (CSPF) on nutrient digestibility and production responses of early-lactation dairy cows grazing on tropical pastures and to evaluate carryover effects throughout mid and late lactation. Forty multiparous dairy cows (Jersey × Holstein) with (mean ± standard error of the mean) 20 ± 1.69 kg of milk/d and 20 ± 5.0 d in milk were used in a randomized complete block design. During the treatment period, all cows were kept in a grazing system. The treatments were offered for 90 d (treatment period) and consisted of 4 increasing levels of CSPF: 0 (0 kg/d), 0.2 (0.2 kg/d), 0.4 (0.4 kg/d), and 0.6 (0.6 kg/d). Each treatment had 10 animals. Increasing CSPF from 0 to 0.6 kg/d replaced an equivalent amount of a corn-based concentrate supplement offered at 10 kg/d on an as-fed basis (8.96 kg/d as a dry matter basis). All cows were housed and received a diet without fat inclusion fed as total mixed ration once a day from 91 to 258 d of the experiment (carryover period). During the treatment period, increasing CSPF linearly decreased dry matter intake (1.20 kg/d), linearly increased neutral detergent fiber digestibility (3.90 percentage units), and quadratically increased total fat digestibility (6.30 percentage units at 0.4 kg/d CSPF). Increasing CSPF linearly increased the yields of milk (4.10 kg/d), milk fat (0.11 kg/d), milk lactose (0.19 kg/d), energy-corrected milk (ECM; 3.30 kg/d), and feed efficiency (ECM/dry matter intake, 0.34 kg/kg), and linearly decreased milk protein content (0.38 g/100 g), body weight change (0.05 kg/d), and body condition score (0.37). We observed interactions between CSPF and time during the carryover period. Overall, CSPF supplementation linearly increased or tended to increase milk yield until 202 d of the experiment with a similar pattern observed for all the other yield variables. In conclusion, supplementing CSPF from 0 to 0.6 kg/d during 90 d increased neutral detergent fiber and total fat digestibility and the yields of milk, milk fat, and ECM in early-lactation dairy cows grazing on tropical pastures. Most production measurements linearly increased during the treatment period, indicating that 0.6 kg/d CSPF was the best dose. Also, supplementing CSPF from 0 to 0.6 kg/d for 90 d during early lactation had positive carryover effects across mid and late lactation.
Key words: carryover effect, fat supplement, grazing system, tropical pasture

INTRODUCTION

During early lactation, the energy intake of dairy cows cannot meet their requirements to support milk production and other metabolic functions (NRC, 2001). This state of negative energy balance can be greater in dairy cows grazing on tropical pastures compared with cows receiving a TMR diet (Muller and Fales, 1998; de Souza et al., 2017). Although adequate grazing management practices have substantially enhanced the nutritional value of tropical grasses, even well managed tropical pastures still impose limitations to energy intake and milk production of high genetic merit dairy cows. As a result, high energy supplements often need to be provided in the diet as a means to improve energy intake (da Silva et al., 2013; Batistel et al., 2017; de Souza et al., 2017).

Corn grain is the main feedstuff supplement used for grazing dairy cows (Bargo et al., 2003; Schroeder et al., 2004), but feeding supplemental fat to grazing animals has some additional advantages, such as the reduced risk of acidosis, higher energy density (Schroeder et al., 2004), improved feed efficiency, and lower environmental impact (Batistel et al., 2021). However, research on

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grazing cows fed supplemental fat is scarce, and it is mainly based on evaluations that consider fatty acids (**FA**) from oilseeds and fat supplements with low digestibility (Weiss and Wyatt, 2004; Schroeder et al., 2004).

Calcium salts of palm fatty acids (**CSPF**) are one of the most common rumen inert fats used in dairy cow diets and primarily contain palmitic (C16:0; ~46%) and oleic (*cis*-9 C18:1; ~38%) acids (dos Santos Neto et al., 2021a). Compared with a prilled saturated triglyceride supplement, the addition of CSPF increased nutrient digestibility and milk yield of lactating dairy cows receiving TMR (Oyebade et al., 2020). Garnsworthy (1990) observed that replacing a starch-based concentrate with CSPF increased milk fat content and yield in cows grazing on temperate pastures. Previous studies with early-lactation dairy cows grazing on tropical pastures observed that replacing a corn-based concentrate with 0.4 kg/d of CSPF increased the yields of milk, milk fat, 3.5% FCM, and ECM from 3 to 16 wk postpartum with a positive carryover effect up until 30 wk postpartum (Batistel et al., 2017; de Souza et al., 2017). Despite these positive effects, to our knowledge, no studies have evaluated a dose response of CSPF in early-lactation dairy cows grazing on tropical pastures. Considering that most dairy farms that use supplemental fat would include it in diets within the range of 0.5 to 3.0% of ration DM, determining an optimal dose has important application.

Therefore, our objective was to determine the dose-response effects of CSPF on nutrient digestibility and production responses of early-lactation dairy cows grazing on tropical pastures and evaluate carryover effects throughout mid and late lactation. We hypothesized the inclusion of CSPF replacing a corn-based supplement would increase NDF and total fat digestibility, the yields of milk, milk fat, 3.5% FCM, and ECM in early-lactation dairy cows with a positive carryover effect across mid and late lactation.

MATERIALS AND METHODS

Animal Care

This study was conducted in Piracicaba, São Paulo, Brazil (22.7°S, 47.6°E, and 546 m altitude) on the dairy facilities at the University of São Paulo, campus Luiz de Queiroz, College of Agriculture (USP-ESALQ). Humane animal care and handling procedures were followed as required by the Ethical Committee for Animal Research (CEUA, protocol number 2017.5.1178.11.9). This study took place between the end of 2017 and the middle of 2018 and is divided into a treatment and carryover period. The treatment period was during the wet season when pastures are fully available for grazing (usually from mid to late spring and throughout the summer in southeastern Brazil). During the carryover period, animals were housed in a free stall and received a TMR diet. The TMR was used due to the seasonal variation because the carryover period partially occurred during the driest seasons when pastures are not fully available for grazing (fall to winter in southeastern Brazil).

Design and Treatments

All animals received a diet with no fat supplementation during a 15 d preliminary period after calving to obtain baseline values of milk yield, BW, and BCS. Forty multiparous dairy cows (Jersey × Holstein) with (mean ± SEM) 489 ± 14.5 kg of BW, 20 ± 1.69 kg of milk/d, and 20 ± 5.0 DIM were used in a randomized complete block design and blocked by milk yield and BCS (10 blocks). All cows were in a grazing system during the treatment period. The treatments were offered for 90 d (treatment period) and consisted of 4 increasing levels of CSPF (Nutri Gordura Lac, Nutricorp Inc.): 0 (0 kg/d), 0.2 (0.2 kg/d), 0.4 (0.4 kg/d), and 0.6 (0.6 kg/d). Increasing CSPF from 0 to 0.6 kg/d replaced an equivalent amount of a corn-based concentrate supplement offered at 10 kg/d on an as-fed basis (8.96 kg/d on a DM basis). Each treatment had 10 animals. Treatments were fed individually, using a tiestall facility in 2 equal feedings 30 min before milking at 0330 and 1530 h, and orts were collected when needed. At the end of the treatment period, all cows were housed in a freestall and received a diet without fat inclusion fed as TMR once a day at 0900 h from 91 to 258 d of the experiment (carryover period). Cows were fed the TMR diet at 110% of expected intake with orts adjusted daily. The ingredients and nutrient composition are shown in Table 1.

Grazing Management

During the treatment period, all cows grazed elephant grass (*Pennisetum purpureum* L. Cameroon) pastures as 1 herd, in an area of 5.0 ha, divided into 0.2-ha paddocks with free access to natural shade and fresh water. The elephant grass was managed in a rotational system based on a canopy height target of 100 cm at entry height. At a canopy height of 100 cm, this elephant grass cultivar has a 95% rate of light interception, which results in maximum net leaf accumulation, minimum stem and dead material accumulation, and high leaf-stem ratio (Congio et al., 2018). Postgrazing target corresponded to approximately 50% of the entry height target (50 cm). The paddocks were fertilized

Table 1. Ingredient and nutrient composition of herbage and treatments

Item	Herbage[1]	CSPF[2] 0	0.2	0.4	0.6	Carryover diet
Ingredient, % DM						
Ground corn		80.0	78.3	76.5	74.8	20.8
Soybean meal		15.0	14.7	14.3	14.0	16.8
Mineral and vitamin mix[3]		5.00	4.89	4.78	4.67	2.00
Calcium salts of palm fatty acids supplement[4]		0.00	2.17	4.34	6.50	
Corn silage						60.0
Urea						0.40
Nutrient composition, % DM						
OM	88.9	91.4	91.0	90.7	90.4	94.3
NDF	56.8	15.8	15.5	15.1	14.8	35.4
ADF	32.4	3.30	3.20	3.10	3.00	17.5
Lignin	3.38	0.13	0.13	0.11	0.10	2.35
CP	20.0	12.2	11.9	11.7	11.4	16.9
Ash	11.1	8.61	8.95	9.29	9.63	5.67
Total fat	4.10	3.42	5.04	6.66	8.27	2.32
12:0	0.04	0.01	0.07	0.14	0.21	
14:0	0.01	0.01	0.04	0.07	0.10	
16:0	0.88	0.81	1.56	2.30	3.04	
18:0	0.09	0.13	0.20	0.27	0.34	
cis-9 18:1	0.06	1.31	1.94	2.57	3.20	
cis-9,*cis*-12 18:2	0.43	1.14	1.25	1.36	1.46	
cis-9,*cis*-12,*cis*-15 18:3	2.06	0.02	0.02	0.02	0.03	

[1]Elephant grass (*Pennisetum purpureum* L. Cameroon).

[2]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d).

[3]Provided 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 D_3, 1,250 of vitamin E.

[4]Calcium salts of palm fatty acids (Nutri Gordura Lac, Nutricorp Inc.). Contained (g/100 g of fatty acids) C12:0 = 3.83, C14:0 = 1.72, C16:0 = 44.0, C18:0 = 4.16, *cis*-9 C18:1 = 38.0, *cis*-9,*cis*-12 C18:2 = 7.56, *cis*-9,*cis*-12,*cis*-15 C18:3 = 0.24; 78% total fatty acids).

with 60 kg of N/ha after each grazing period (Batistel et al., 2017; de Souza et al., 2017). Cows were switched to a new paddock every day after evening milkings, and the average grazing interval was 22.5 ± 1.4 d. When the postgrazing height of the paddock was not reached, a group of dry cows was used to graze down the pasture to the target postgrazing height (50 cm).

Data and Sample Collection

During the treatment period, pre- and postgrazing heights were measured every day at 20 randomized points before the animals entered as well as after they left the paddock (Table 2). Pre- and postgrazing herbage mass were measured in 2 paddocks every 7 d by clipping the herbage inside a rectangular frame (0.94 m^2) at 3 cm above ground level from sites that represented the mean sward height of the paddock. The total herbage mass was weighed, and 2 representative subsamples (500 g) were 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 (including leaves sheaths), and senescent material (as indicated by more than 50% of the tissue area being senescent with either a typically yellowish or brownish color), and then dried in a forced-air oven at 55°C for 72 h to determine the morphological composition of the herbage mass (Table 2, de Souza et al., 2017; Congio et al., 2018).

Herbage and concentrate ingredients were collected for determination of their chemical composition every 7 d. Handpicked herbage samples were taken at 20 randomized points before cows entered the paddock by simulating the cows' grazing habits as described by De Vries (1995). All samples were dried in a forced-air oven at 55°C for 72 h and ground through a 1-mm screen (Wiley mill, Thomas Scientific).

Nutrient intake and digestibility were measured 3 times during the study through total fecal estimation using TiO_2 and the indigestible NDF content of feces and feeds (de Souza et al., 2015; Batistel et al., 2017; Congio et al., 2018). For 15 d in each intake estimation period (period 1: from 15 to 29 d; period 2: from 45 to 59 d; and period 3: from 75 to 89 d of the experiment), cows received a daily dose of TiO_2 (20 g/cow per d) where we used balling guns to deliver capsules containing half of the total dose (10 g) before each milking and immediately before offering the treatments. In the last 5 d of each period, fecal grab samples were collected directly from the rectum after morning and afternoon

Table 2. Herbage characteristics at pre- and postgrazing

Item	Pregrazing	SD	Postgrazing	SD
Sward height, cm	98.2	2.35	56.9	2.90
Herbage mass, kg of DM/ha	8,089	912	4,551	485
Daily herbage allowance,[1] kg of DM/cow per d	40.4	4.56	—	—
Morphological composition, % of forage DM				
Leaves	50.0	8.99	24.6	16.2
Stem	36.2	4.55	47.7	4.70
Senescent material	12.8	1.38	27.7	6.42
Leaf-stem ratio	1.38	0.89	0.52	0.18

[1]Daily herbage allowance = [pregrazing herbage mass (kg of DM/ha) × paddock area (ha)]/number of cows, where pregrazing herbage mass = 8,089 kg of DM/ha, paddock area = 0.2 ha, and number of cows = 40/d.

milkings, and immediately stored at −20°C. Samples were subsequently thawed, dried at 55°C for 72 h in a forced-air oven, and ground through a 1 mm screen (Wiley mill, Thomas Scientific). Fecal samples were composited, forming 1 sample per cow that was used for analyses as described below. During the last 5 d of each intake estimation period, herbage and concentrate ingredients were collected daily to determine nutrient intake and nutrient digestibility following the procedures described below. We did not observe orts when offering the treatments during the intake estimation periods.

Cows were milked twice a day at 0400 and 1600 h. Milk yield was recorded, and milk samples from both milkings were collected every 7 d for milk component analysis (concentrations of fat, true protein, lactose, and MUN) and preserved with a bronopol preservative pill (Advanced Instruments). Milk samples used to determine milk FA profile were collected without preservative on 45 and 90 d of the experiment and stored at −20°C. The cows were weighed every 7 d after morning milking and scored for BCS at the same time by 3 trained investigators on a scale from 1 to 5 points in 0.25 point increments (Wildman et al., 1982).

During the carryover period (91 to 258 d of the experiment), feed ingredients of the TMR 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 to determine diet composition. We measured milk yield, milk components (concentrations of fat, true protein, lactose, and MUN), BW, and BCS during the carryover period following the same collection procedures described in the treatment period. It is noteworthy that we did not collect samples to determine the milk FA profile. Dry matter intake was estimated twice during the carryover period from total fecal excretion and feed indigestibility. For 15 d in each intake estimation period (period 1: from 121 to 135 d; and period 2: from 166 to 180 d), cows received a daily dose of TiO_2 (20 g/cow per d). During the last 5 d of each intake estimation period, feed ingredients were collected daily to determine nutrient intake. All the procedures to estimate intake occurred as described in the treatment period. We also used the tiestall facility to quickly deliver half of the total TiO_2 dose (10 g) before each milking.

Sample Analysis and Calculations

Herbage allowance was calculated using the formula: daily herbage allowance = [pregrazing herbage mass (kg of DM/ha) × paddock area (ha)]/number of cows where pregrazing herbage mass = 8,089 kg of DM/ha, paddock area = 0.2 ha, and number of cows = 40/d (Table 2).

Herbage intake was estimated from total fecal excretion and feed indigestibility. To estimate fecal excretion, fecal samples were analyzed for Ti concentration according to Myers et al. (2004). The indigestible NDF contents of the herbage, concentrate, and fecal samples (NDF remaining after 240 h of in situ incubation; Huhtanen et al., 1994) were determined to calculate indigestibility. Total fecal excretion and herbage intake were calculated according to de Souza et al. (2015).

Herbage, concentrate, and fecal samples were analyzed for DM by drying samples in an oven at 105°C for 24 h (AOAC International, 2005; method 942.05), total N content by the Dumas combustion method using N analyzer and CP calculated as N × 6.25 (Leco FP-2000 N Analyzer; Leco Instruments Inc.), NDF with sodium sulfite and heat-stable α-amylase (Van Soest et al., 1991), ADF and lignin determined by solubilization with sulfuric acid (AOAC International, 2005; method 973.18), and total fat determined by Soxhlet extraction with petroleum ether and glacial acetic acid (Sukhija and Palmquist, 1988).

Milk samples were analyzed for fat, protein, lactose, and MUN using infrared procedures (Foss 4000; Foss North America). Milk component yields were calculated from milk component contents for each milking and summed for a daily total. Yields of 3.5% FCM and ECM were calculated using the yields of milk and milk components as follows: 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)] (NRC, 2001), and ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (based on Tyrrell and Reid, 1965). We used the overall averages of ECM and DMI during the treatment period to calculate feed efficiency as ECM/DMI (kg/kg). We could match the weekly ECM measurements with the 3 DMI measurements by using these overall treatment period averages.

Diet lipids were extracted according to Folch et al. (1957) and milk lipids were extracted according to Feng et al. (2004). The separated fat was methylated according to Kramer et al. (1997). The FAME were quantified by gas chromatograph (GC Shimatzu 2010 with automatic injection, Shimadzu Corporation) fitted with a flame-ionization detector and equipped with a SP-2560 capillary column (100 m × 0.25 mm i.d. with a 0.02-μm film thickness, Supelco). Hydrogen was used as the carrier gas (40 cm^3/s flow rate). A more detailed description of this method was reported by Marques et al. (2019).

During calculations, we did not use the response factors published by Ulberth and Schrammel (1995) to correct the mass discrepancy of short-chain FA such as C4:0 and C6:0. Therefore, their content and yield average values might be somewhat low regardless of treatment, but it does not affect the interpretation of our results. Yields of individual FA (g/d) in milk fat were calculated by using milk fat yield and individual FA concentration, correcting milk fat yield for glycerol content and other milk lipid classes (Piantoni et al., 2013). We calculated the summation of milk FA concentrations and yields by source (de novo [$\Sigma < $ C16], mixed [Σ C16 + C16:1], and preformed [$\Sigma >$ C16]).

Mean daily BW change (kg/d) was calculated for each cow monthly by a linear regression applied to BW measurements where the resulting slope represents the BW change (Boerman et al., 2015a). Energy intake and energy concentration of the diet were calculated as follows: digestible energy (**DE**) = 0.04 × TDN (estimated from nutrient digestibility), metabolizable energy (ME) = [(1.01 × DE) − 0.45], net energy (NE_L) = [(ME × 0.703) − 0.19], and diet NE_L = NE_L/DMI. Energy intake (Mcal/d) was calculated for each cow from NE_L × DMI (NRC, 2001; Boerman et al., 2015a). Energy output (Mcal/d) to milk, body reserves, and maintenance was calculated monthly according to NRC (2001): milk energy output (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)]. Body reserves output (Mcal/d) = [(2.88 + 1.036 × BCS) × ΔBW], where ΔBW is BW change (Boerman et al., 2015a), and energy output to maintenance (Mcal/d) = 0.08 Mcal/kg × BW $(kg)^{0.75}$.

During the carryover period, we performed the same analyses and calculations as those reported during the treatment period, except for feed efficiency, nutrient digestibility, energy output, and milk FA profile.

Statistical Analysis

Data were analyzed separately for the treatment (90 d of supplementation) and carryover (from 91 to 258 d) periods. The measurements taken during the 15 d of the preliminary period were tested as covariates but were not used in the final statistical model because they were not significant. All data were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc.). Data were analyzed with repeated measures according to the following model:

$$Y_{ijkl} = \mu + F_i + T_j + B_k + C_l(F_i\,B_k) + F_i \times T_j + e_{ijkl},$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, F_i = the fixed effect of treatment, T_j = the fixed effect of time, B_k = the random effect of block, $C_l(F_i\,B_k)$ = the random effect of cow within treatment and block, $F_i \times T_j$ = the fixed effect of the interaction between treatment and time, and e_{ijkl} = the residual error. First-order autoregressive was the covariance structure used for analysis because it resulted in the smallest Akaike's information criteria (AICC) for most of the variables measured. In addition, this covariance structure is ideal for repeated measurements with equally spaced time points (Littell et al., 2006). Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Three preplanned contrasts were used to determine the linear, quadratic, and cubic effects of increasing CSPF, but we also reported the interactions between treatment and time (de Souza et al., 2021). When the interaction between CSPF treatment × time tended to be significant ($P \leq 0.10$), these 3 preplanned contrasts were used to determine the effects of increasing CSPF over time. Significant differences were declared at $P \leq 0.05$, and tendencies at $P \leq 0.10$ for main effects and interactions.

RESULTS

Nutrient Intake and Total-Tract Digestibility During Treatment Period

Increasing CSPF from 0 to 0.6 kg/d had no effect on DM digestibility ($P \geq 0.14$) or CP digestibility ($P \geq 0.67$). Increasing CSPF from 0 to 0.6 kg/d linearly decreased the intakes of herbage (1.20 kg/d, $P = 0.02$), DMI (1.20 kg/d), OM (1.00 kg/d), NDF (0.70 kg/d, $P =$

Table 3. Nutrient intake and digestibility of cows fed treatment diets (n = 40)

Item	CSPF[1]				SEM	Contrast[2]			P-value[3]	
	0	0.2	0.4	0.6		Linear	Quadratic	Cubic	Time	Trt × Time
Intake, kg/d										
Herbage	8.24	7.28	7.09	7.04	0.36	0.02	0.22	0.71	<0.01	0.16
DM	17.2	16.3	16.1	16.0	0.36	0.03	0.21	0.70	<0.01	0.16
OM	15.4	14.6	14.5	14.4	0.32	0.03	0.24	0.60	<0.01	0.37
NDF	6.03	5.45	5.42	5.33	0.21	0.03	0.25	0.52	<0.01	0.72
CP	2.69	2.47	2.45	2.41	0.07	0.01	0.25	0.51	<0.01	0.75
Total fat	0.74	0.81	0.88	1.00	0.02	<0.01	0.17	0.42	<0.01	<0.01
Digestibility, %										
DM	65.4	66.9	67.1	67.0	0.82	0.14	0.33	0.82	0.79	0.38
OM	70.0	71.6	73.1	71.7	1.03	0.06	0.06	0.44	0.08	0.14
NDF	52.5	54.9	55.4	56.4	1.51	0.02	0.53	0.63	<0.01	0.71
CP	64.9	64.9	64.5	64.4	1.20	0.67	0.92	0.85	0.02	0.32
Total fat	72.1	75.1	78.4	75.3	1.17	<0.01	<0.01	0.11	<0.01	0.85

[1]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals.

[2]*P*-values associated with contrasts: (1) the linear effect of increasing CSPF, (2) the quadratic effect of increasing CSPF, and (3) the cubic effect of increasing CSPF.

[3]*P*-values associated with the effects of time, and treatment (CSPF) × time.

0.03), and CP (0.28 kg/d, $P = 0.01$), linearly increased total fat intake (0.26 kg/d, $P < 0.01$) and NDF digestibility (3.90 percentage units, $P = 0.02$), quadratically increased total fat digestibility (6.30 percentage units, $P < 0.01$), and tended to quadratically increase OM digestibility ($P = 0.06$) with a maximum value at 0.4 g/d kg CSPF (Table 3). We observed an interaction between treatment and time for total fat intake ($P <$ 0.01, Table 3), but the pattern of the responses was the same within months with CSPF linearly increasing total fat intake at mo 1, 2, and 3 ($P < 0.01$, sliced interactions not shown).

Production Responses During Treatment Period

Increasing CSPF from 0 to 0.6 kg/d linearly increased the yields of milk (4.10 kg/d), milk fat (0.11 kg/d), milk lactose (0.19 kg/d), 3.5% FCM (3.80 kg/d), ECM (3.30 kg/d), and feed efficiency (ECM/DMI, 0.34 kg/kg, $P <$ 0.01), and linearly decreased milk protein content (0.38 g/100 g, $P < 0.01$), BW change (0.05 kg/d, $P < 0.01$), and BCS (0.37, $P = 0.04$, Table 4). Interactions between treatment and time were observed for BW change ($P =$ 0.01) and BCS ($P < 0.01$, Table 4). Increasing CSPF from 0 to 0.6 kg/d cubically decreased BW change at mo 1 ($P < 0.01$), linearly decreased at mo 2, and cubically decreased at mo 3 ($P < 0.01$, Supplemental Figure S1; https://doi.org/10.5281/zenodo.7129335). Increasing CSPF linearly decreased BCS from 15 to 50 d ($P \leq 0.04$) and cubically decreased or tended to decrease it from 57 to 78 d ($P \leq 0.08$) except on 71 d of the experiment ($P = 0.71$, Supplemental Figure S2; https://doi.org/10.5281/zenodo.7129335). Increasing CSPF had no effects on milk protein yield ($P \geq 0.22$), milk fat content ($P \geq 0.55$), milk lactose content ($P \geq 0.21$), MUN ($P \geq 0.14$), or BW ($P \geq 0.66$, Table 4).

Energy Intake and Output

Increasing CSPF from 0 to 0.6 kg/d had no linear, quadratic, or cubic effect on NE_L intake ($P \geq 0.66$) or energy output to maintenance ($P \geq 0.63$), linearly increased diet NE_L (0.08 Mcal/d, $P < 0.01$) and energy output to milk (2.10 Mcal/d, $P = 0.01$), and linearly decreased energy output to body reserves (0.34 Mcal/d, $P < 0.01$, Table 5). We observed an interaction between treatment and time for energy output to body reserves ($P = 0.01$, Table 5), whose pattern was similar to that observed for BW change. Increasing CSPF cubically decreased energy output to body reserves at mo 1 (P < 0.01), linearly decreased at mo 2, and cubically decreased at mo 3 ($P < 0.01$, Supplemental Figure S1).

Milk Fatty Acid Concentration and Yield During Treatment Period

Milk FA are derived from 2 sources: <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from plasma extraction. Mixed source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and extraction from plasma. We did not observe interactions between CSPF and time for the yield or content of milk FA by source ($P \geq 0.27$, Table 6). Increasing CSPF from 0 to 0.6 kg/d linearly decreased the yield of de novo milk FA (36.0 g/d, Table 6) primarily due to decreased C8:0 (1.68 g/d), C10:0 (4.30 g/d), C12:0 (5.00 g/d, $P =$ 0.01), C14:0 (13.7 g/d, $P = 0.02$), and C14:1 (1.73 g/d,

Table 4. Milk yield, milk composition, BW, and BCS of cows fed treatment diets (n = 40)

Item	CSPF[1]				SEM	Contrast[2]			P-value[3]	
	0	0.2	0.4	0.6		Linear	Quadratic	Cubic	Time	Trt × Time
Yield, kg/d										
Milk	23.0	24.1	25.5	27.1	1.63	<0.01	0.80	0.95	<0.01	0.58
Fat	0.75	0.78	0.82	0.86	0.05	<0.01	0.90	0.92	<0.01	0.99
Protein	0.73	0.74	0.76	0.78	0.05	0.22	0.92	0.96	<0.01	0.81
Lactose	1.07	1.13	1.17	1.26	0.07	<0.01	0.74	0.67	<0.01	0.79
3.5% FCM[4]	22.0	23.1	24.1	25.8	1.47	<0.01	0.74	0.84	<0.01	0.66
ECM[5]	22.4	23.4	24.3	25.7	1.48	<0.01	0.79	0.89	<0.01	0.68
ECM/DMI,[6] kg/kg	1.28	1.43	1.53	1.62	0.09	<0.01	0.57	0.94	—	—
Content, g/100 g										
Fat	3.33	3.34	3.36	3.25	0.10	0.61	0.55	0.76	0.06	0.65
Protein	3.20	3.05	3.00	2.82	0.07	<0.01	0.79	0.42	<0.01	0.27
Lactose	4.66	4.64	4.60	4.56	0.06	0.21	0.87	0.94	<0.01	0.32
MUN, mg/dL	14.0	13.6	13.2	13.1	0.57	0.14	0.76	0.84	<0.01	0.99
BW, kg	501	490	496	494	14.4	0.78	0.67	0.66	<0.01	0.18
BW change, kg/d	0.12	0.09	0.09	0.07	0.01	<0.01	0.20	0.25	<0.01	0.01
BCS	3.06	2.90	2.85	2.69	0.13	0.04	0.99	0.69	<0.01	<0.01

[1]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals.

[2]P-values associated with contrasts: (1) the linear effect of increasing CSPF, (2) the quadratic effect of increasing CSPF, and (3) the cubic effect of increasing CSPF.

[3]P-values associated with the effects of time, and treatment (CSPF) × time.

[4]3.5% FCM = (0.4324 × kg milk) + (16.216 × kg milk fat).

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

[6]We used the ECM and DMI average during the treatment period.

$P = 0.01$, Supplemental Table S1; https://doi.org/10.5281/zenodo.7129335). Interestingly, increasing CSPF linearly increased the yield of C4:0 (3.10 g/d, $P < 0.01$) and had no linear, quadratic, or cubic effect on the yield of C6:0 ($P \geq 0.64$, Supplemental Table S1). Increasing CSPF from 0 to 0.6 kg/d linearly increased the yield of mixed milk FA (65.0 g/d, Table 6) primarily due to increased C16:0 (64.0 g/d, $P < 0.01$, Supplemental Table S1), and increased the yield of preformed milk FA (60 g/d, Table 6) primarily due to increased total *trans*-C18:1 (7.90 g/d), *cis*-9 C18:1 (42.0 g/d), *cis*-9,*cis*-12 C18:2 (3.90 g/d), and *cis*-9,*trans*-11 C18:2 (1.29 g/d, $P < 0.01$, Supplemental Table S1). We observed a similar pattern of results for the inclusion of CSPF on a content basis (g/100 g) compared with a yield basis (g/d; Table 6, Supplemental Table S1).

Table 5. Energy intake and output of cows fed treatment diets (n = 40)

Item	CSPF[1]				SEM	Contrast[2]			P-value[3]	
	0	0.2	0.4	0.6		Linear	Quadratic	Cubic	Time	Trt × Time
Energy intake,[4] Mcal/d										
NE_L	27.5	27.1	27.3	27.0	0.56	0.66	0.91	0.70	<0.01	0.45
Diet NE_L	1.61	1.66	1.70	1.69	0.03	<0.01	0.18	0.70	0.03	0.26
Energy output, Mcal/d										
Milk[5]	15.5	15.9	16.9	17.6	0.98	0.01	0.82	0.77	0.02	0.34
Body reserves[6]	0.74	0.54	0.50	0.40	0.04	<0.01	0.12	0.17	<0.01	0.01
Maintenance[7]	8.47	8.34	8.40	8.39	0.16	0.75	0.64	0.63	<0.01	0.18

[1]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals.

[2]P-values associated with contrasts: (1) the linear effect of increasing CSPF, (2) the quadratic effect of increasing CSPF, and (3) the cubic effect of increasing CSPF.

[3]P-values associated with the effects of time, and treatment (CSPF) × time.

[4]Digestible energy (DE) = 0.04 × TDN (estimated from nutrient digestibility) ME = (1.01 × DE) – 0.45; NE_L = (ME × 0.703) – 0.19; diet NE_L = NE_L/DMI (NRC, 2001; Boerman et al., 2015a).

[5]From NRC (2001): milk energy output (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)].

[6]From NRC (2001) according to Boerman et al. (2015a): body reserves output (Mcal/d) = [(2.88 + 1.036 × BCS) × ΔBW], where BCS was the average BCS, and ΔBW was BW change.

[7]From NRC (2001): energy output for maintenance (Mcal/d) = 0.08 Mcal/kg × BW $(kg)^{0.75}$.

Table 6. Fatty acid (FA) concentration and yield by source of milk FA of cows fed treatment diets (n = 40)

Item	CSPF[1]				SEM	Contrast[2]			P-value[3]	
	0	0.2	0.4	0.6		Linear	Quadratic	Cubic	Time	Trt × Time
Summation by source,[4] g/d										
De novo	167	153	141	131	10.7	<0.01	0.82	0.97	0.32	0.27
Mixed	234	255	269	299	18.6	<0.01	0.76	0.72	0.63	0.32
Preformed	324	327	361	384	24.1	<0.01	0.55	0.58	<0.01	0.73
Summation by source,[4] g/100 g										
De novo	21.6	19.8	17.4	16.2	0.60	<0.01	0.56	0.47	0.02	0.39
Mixed	30.8	32.6	33.6	34.8	0.84	<0.01	0.73	0.73	<0.01	0.77
Preformed	42.2	43.3	44.7	46.1	0.99	<0.01	0.94	0.93	0.03	0.79

[1]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals.

[2]*P*-values associated with contrasts: (1) the linear effect of increasing CSPF, (2) the quadratic effect of increasing CSPF, and (3) the cubic effect of increasing CSPF.

[3]*P*-values associated with the effects of time, and treatment (CSPF) × time.

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

Dry Matter Intake and Production Responses During Carryover Period

Overall, supplementing CSPF from 0 to 0.6 kg/d until 90 d of the experiment had no effect on DMI ($P \geq 0.12$), milk component contents ($P \geq 0.11$), BW ($P \geq 0.76$), BW change ($P \geq 0.11$), or BCS ($P \geq 0.46$), tended to linearly increase the yields of milk ($P = 0.09$) and milk protein ($P = 0.06$), linearly increase the yields of milk fat (0.16 kg/d, $P < 0.01$) and milk lactose (0.17 kg/d, $P = 0.02$), and quadratically increased 3.5% FCM (4.20 kg/d, $P = 0.02$) and ECM (4.00 kg/d, $P = 0.01$) during the carryover period (Table 7). Even with a quadratic response, the maximum values for 3.5% FCM and ECM were obtained with the highest dose, at 0.6 kg/d CSPF.

We observed interactions between CSPF and time during the carryover period for the yields of milk, milk fat, milk protein, milk lactose, 3.5% FCM, and ECM ($P < 0.01$, Table 7). Increasing CSPF from 0 to 0.6 kg/d for 90 d during the treatment period linearly increased ($P \leq 0.05$) or tended to increase ($P \leq 0.10$) milk yield

Table 7. Dry matter intake, milk yield, and milk composition of cows during carryover period (n = 40)

Item	CSPF[1]				SEM	Contrast[2]			P-value[3]	
	0	0.2	0.4	0.6		Linear	Quadratic	Cubic	Time	Trt × Time
DMI	22.0	20.6	21.4	21.9	0.61	0.83	0.12	0.42	0.23	0.91
Yield, kg/d										
Milk	16.7	17.1	18.2	20.6	1.73	0.09	0.56	0.96	<0.01	<0.01
Fat	0.65	0.67	0.71	0.81	0.02	<0.01	0.09	0.81	<0.01	<0.01
Protein	0.60	0.61	0.64	0.71	0.03	0.06	0.38	0.91	<0.01	<0.01
Lactose	0.74	0.75	0.80	0.91	0.04	0.02	0.24	0.89	<0.01	<0.01
3.5% FCM[4]	17.8	18.2	19.4	22.0	0.35	<0.01	0.02	0.72	<0.01	<0.01
ECM[5]	18.3	18.6	19.8	22.3	0.39	<0.01	<0.01	0.53	<0.01	<0.01
Content, g/100 g										
Fat	4.21	4.21	4.20	4.23	0.10	0.88	0.89	0.91	<0.01	0.39
Protein	3.65	3.61	3.55	3.51	0.20	0.58	0.99	0.97	<0.01	0.99
Lactose	4.45	4.42	4.41	4.44	0.03	0.82	0.40	0.86	0.34	0.99
MUN, mg/dL	15.4	15.2	14.1	14.2	0.81	0.45	0.11	0.93	0.11	0.93
BW, kg	546	539	540	538	24.5	0.76	0.76	0.90	0.03	0.35
BW change, kg/d	0.21	0.21	0.23	0.23	0.08	0.11	0.58	0.19	0.02	0.28
BCS	3.41	3.32	3.28	3.24	0.18	0.46	0.85	0.94	0.27	0.59

[1]Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals.

[2]*P*-values associated with contrasts: (1) the linear effect of increasing CSPF, (2) the quadratic effect of increasing CSPF, and (3) the cubic effect of increasing CSPF.

[3]*P*-values associated with the effects of time and the interaction treatment (CSPF) × time.

[4]3.5% FCM = (0.4324 × kg milk) + (16.216 × kg milk fat).

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

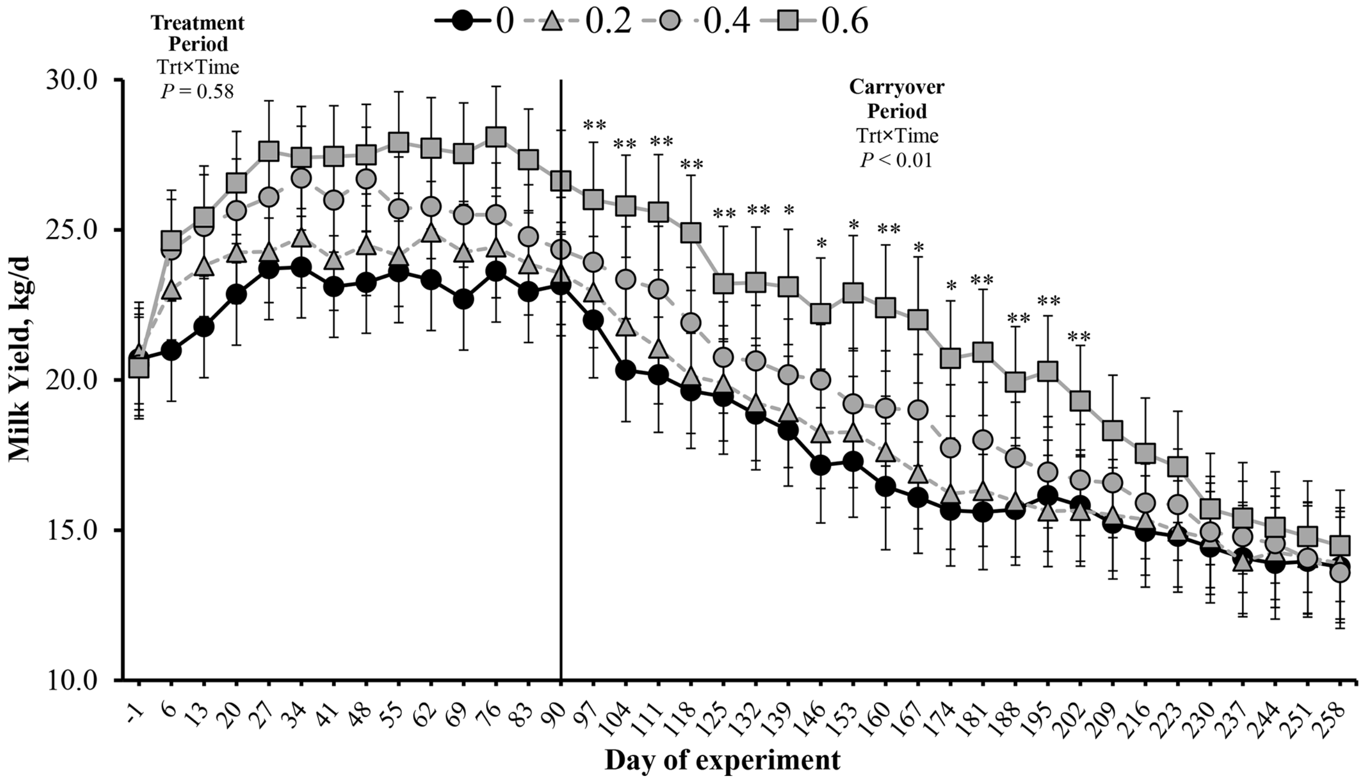

Figure 1. Milk yield over time during treatment and carryover period (n = 40). Inclusion of 4 levels of calcium salts of palm fatty acids (CSPF) replacing a corn-based supplement: (1) 0 (0 kg/d), (2) 0.2 (0.2 kg/d), (3) 0.4 (0.4 kg/d), and (4) 0.6 (0.6 kg/d). Each treatment had 10 animals. Trt × Time = P-value associated with the interaction treatment (CSPF) × time during treatment and carryover period. Linear effects with tendencies at $^{*}P \leq 0.10$; and significances at $^{**}P \leq 0.05$. Error bars represent SEM.

until 202 d of the experiment (Figure 1). Overall, all the other yield variables had a similar pattern to that observed for milk yield (data not shown).

DISCUSSION

Information on the impact of fat supplementation in grazing dairy cows is limited. Previous studies have shown that CSPF can increase the yields of milk and milk fat of early-lactation dairy cows grazing on tropical pastures (Batistel et al., 2017; de Souza et al., 2017), but the dose response of CSPF is not well established. Therefore, our objective was to evaluate the effect of increasing doses of CSPF on nutrient digestibility and production responses of early-lactation dairy cows grazing on tropical pastures and whether CSPF supplementation would have carryover effects throughout lactation.

We observed that increasing CSPF from 0 to 0.6 kg/d decreased DMI. In accordance with the literature, a meta-analysis observed that CSPF included at ≤3% of diet DM reduced DMI of lactating dairy cows by 0.56 kg/d (dos Santos Neto et al., 2021a). Other previous meta-analyses have reported that CSPF decreased DMI by 0.40 (Weld and Armentano, 2017), 0.64 (Rabiee et al., 2012), and 0.97 kg/d (Onetti and Grummer, 2004). It is interesting to note that palatability issues did not happen with the supplement and concentrate mix, as we observed no orts during the intake estimation periods. Thus, the decrease in DMI was driven by a reduction in herbage intake, indicating some physiological or metabolic regulation after CSPF feeding. This is likely associated with the secretagogue action of UFA on the release of hormones that inhibit gastric emptying (Allen, 2000; Bradford et al., 2008). Although increasing CSPF linearly decreased DMI, there was no effect on NE_L intake due to the increased diet NE_L content. On the other hand, de Souza et al. (2017) reported that supplementing 0.4 kg/d of CSPF to early-lactation dairy cows grazing on tropical pastures increased NE_L compared with both a nonfat supplemented treatment and a treatment including 0.4 kg/d of calcium salts of soybean oil. Schroeder et al. (2004) summarized the effects of fat supplementation in grazing cows wherein out of 18, only 3 studies had information on herbage intake. Further research is needed to better understand the effects of CSPF supplementation on DMI of grazing dairy cows.

Fat supplementation can decrease fiber digestibility (Devendra and Lewis, 1974; Jenkins and Palmquist, 1984). Most of these negative effects are associated with the dietary inclusion of high fat levels, mainly in the form of pure oils (Ikwuegbu and Sutton, 1982; Rodrigues et al., 2019). On the other hand, we observed that CSPF did not affect DM digestibility, tended to quadratically increase OM digestibility, and linearly increased NDF digestibility. Similarly, de Souza et al. (2017) observed that CSPF increased NDF digestibility of grazing dairy cows. A meta-regression by dos Santos Neto et al. (2021a) reported that each 1 percentage unit increase of CSPF in diet DM increased NDF digestibility by 1.15 percentage units. The increase in NDF digestibility with CSPF is likely associated with decreasing DM and NDF intake. Additionally, although the ionic bonds between calcium ions and FA are satisfactorily stable, some dissociation still occurs even at a rumen pH range of 6.0 to 6.5 (Sukhija and Palmquist, 1990), which provides some free FA to the microorganisms. Palmitic acid is one of the major FA in CSPF (dos Santos Neto et al., 2021a). Recently, C16:0 -enriched supplements have consistently demonstrated improvements in NDF digestibility (de Souza and Lock, 2018; de Souza and Lock, 2019; dos Santos Neto et al., 2021b). It seems C16:0 has a positive effect on fibrolytic bacteria, as this FA is an important component of their biomembrane cells (Mackie et al., 1991; Vlaeminck et al., 2006). Therefore, in our study, CSPF likely increased NDF digestibility by both a decrease in NDF intake and a positive effect of C16:0 on fibrolytic bacteria (dos Santos Neto et al., 2021a). Also, as CSPF replaced a corn-based supplement, the increase in NDF digestibility might be partially explained by a reduced starch intake, possibly improving rumen pH. This topic needs further examination.

Increases in total FA duodenal flow usually decrease FA digestibility (Boerman et al., 2015b). We observed that CSPF had a positive linear effect on total fat intake and quadratically increased total fat digestibility with a maximum value obtained at 0.4 kg of added CSPF. Inclusions of CSPF above that point reduced total fat digestibility. Our results support the hypothesis that up to a certain level, fat digestibility is more affected by the profile of FA entering the duodenum than the total flow of FA in the small intestine (Rico et al., 2017; de Souza et al., 2018). In our study, feeding CSPF likely increased *cis*-9 C18:1 supply to the duodenum. The positive effects of *cis*-9 C18:1 on total fat digestibility are related to its emulsifying properties that increase micellar solubility and the uptake and reesterification of SFA in enterocytes (Freeman, 1969; Ockner et al., 1972). Similar to our results, recent studies observed that FA digestibility increased in response to more dietary *cis*-9 C18:1 (Western et al., 2020; Burch et al., 2021; Prom and Lock, 2021). Prom et al. (2021) reported that abomasally infusing *cis*-9 C18:1 from 0 to 60 g/d linearly increased the digestibility of total, 16-carbon, and 18-carbon FA by 8.40, 8.30, and 8.60 percentage units, respectively. Also, a study comparing different exogenous emulsifiers found that polysorbate-C18:1 improved 18-carbon FA digestibility compared with polysorbate-C16:0 and polysorbate-C18:0+C16:0 (Prom et al., 2022).

Supplementing CSPF from 0 to 0.6 kg/d linearly increased energy output to milk and the yields of milk, milk fat, 3.5% FCM, and ECM. Due to reduced DMI and increased production responses, we also observed that CSPF improved feed efficiency (ECM/DMI). Overall, fat supplementation improves the production responses of lactating dairy cows by generating more ATP per mol than glucose and protein and by sparing energy by decreasing de novo milk FA synthesis (Bauman and Davis, 1974; Palmquist, 1994; Palmquist, 2006). Reducing de novo milk FA yield minimizes the use of NADPH from glucose to generate FA in the mammary gland. This spared glucose can be used in lactose synthesis (Storry et al., 1973; Bauman and Davis, 1974). Indeed, we observed that CSPF linearly decreased de novo milk FA yield while it linearly increased milk lactose yield. Lactose is the main osmotic regulator between the blood and alveolar lumen; therefore, changes observed in lactose yield are also expected to occur in milk yield (Costa et al., 2019). Additional nutritional aspects such as increased NDF and fat digestibility are also related to the positive effects of CSPF on production responses. Schroeder et al. (2004) observed that both saturated and unsaturated fat supplements increased milk yield in dairy cows grazing on temperate pastures. Likewise, supplementing corn oil plus palm kernel oil increased the yields of milk and ECM in mid lactation grazing cows (Parales Girón et al., 2016). The inclusion of CSPF in a corn-based supplement increased the yields of milk, milk fat, and 3.5% FCM in early-lactation dairy cows grazing on tropical pastures (Batistel et al., 2017; de Souza et al., 2017). Similar outcomes have been reported in a meta-analysis that evaluated the effects of CSPF in dairy cows receiving TMR (dos Santos Neto et al., 2021a).

We also observed that including CSPF had no effect on milk protein yield but linearly decreased milk protein content. This dilution effect is a common observation when using CSPF (de Souza et al., 2018; Oyebade et al., 2020; dos Santos Neto et al., 2021a). Furthermore, increasing CSPF linearly decreased energy output to body reserves, BW change, and BCS. In our study, CSPF replaced a starch-based supplement composed of 80% corn. It is well known that gluconeogenic diets have an essential role in modulating energy partitioning toward body reserves in lactating dairy cows by increasing nutrient uptake by muscle and adipose tissue (Bauman and Elliot, 1983; van Knegsel et al., 2007). Previous studies observed similar results when replacing gluconeogenic with lipogenic ingredients in the diets of lactating dairy cows. Boerman et al. (2015a) found that partially replacing starch with fiber and a C16:0-enriched supplement decreased energy output to body reserves. Garnsworthy (1990) observed that feeding grazing cows with CSPF increased energy output to milk and reduced BW change compared with a starch-based supplement. Similarly, Batistel et al. (2017) and de Souza et al. (2017) reported that replacing starch with CSPF decreased BW change and BCS in early-lactation dairy cows grazing on tropical pastures. This topic certainly deserves additional attention due to the negative association between BCS loss and reproductive performance in early-lactation dairy cows (Chagas et al., 2007; Roche et al., 2009). By contrast, Sklan et al. (1991) observed that replacing corn with CSPF from parturition to 120 DIM increased plasma progesterone and pregnancy rate of cows despite the increase in BW loss. Interestedly, replacing soybean hulls with *cis*-9 C18:1 from blends based on CSPF increased BW change in mid lactation cows fed TMR (de Souza et al., 2018, 2019). Further studies are necessary to evaluate how CSPF interact with different diet ingredients on the nutrient partitioning of lactating dairy cows.

Feeding CSPF increased milk fat yield by increasing the yields of mixed and preformed milk FA. Nonetheless, we observed that CPSF linearly reduced the yield of de novo milk FA. In our study, we did not detect levels of *trans*-10,*cis*-12 C18:2, which is the most potent biohydrogenation intermediate that inhibits milk FA synthesis (Bauman et al., 2011). Elevated *trans*-10 C18:1 from the ruminal biohydrogenation of *cis*-9 C18:1 is also associated with reduced de novo FA (Mosley et al., 2002; Dórea and Armentano, 2017). However, Lock et al. (2007) observed that the abomasal infusion of *trans*-10 C18:1 had no direct effect on the yield of de novo milk FA, even when provided at a dose 10 times greater than the effective dose of *trans*-10,*cis*-12 C18:2 (40 vs. 4 g/d). Therefore, CSPF supplementation may have caused a substitution effect between de novo milk FA and exogenous long-chain FA. The mechanisms of FA substitution when feeding CSPF can be explained by FA competition during milk triglyceride synthesis, as proposed in detail by dos Santos Neto et al. (2021a). Similar to that study, we also observed that CSPF decreased the yields of milk FA from 8 to 14 carbons while it increased C16:0 and unsaturated 18-carbon FA. Interestingly, Glasser et al. (2008) suggested an interdependence between the FA in milk fat wherein the synthesis of de novo FA in the mammary gland is stimulated by incorporating long-chain FA from low-fat diets. Partial interdependence has been found in lactating dairy cows receiving diets with a C16:0-enriched supplement that increased the yield of C4:0 (Piantoni et al., 2013; de Souza and Lock, 2018; dos Santos Neto et al., 2021b). Correspondingly, we observed a linear increase in both C16:0 and C4:0. Probably, with the linear increase of C16:0 (melting point of 63.0°C), the mammary gland may be prioritizing the synthesis of C4:0 (melting point of −5.3°C) to help maintain milk fluidity at body temperature (Barbano and Sherbon, 1980; Scrimgeour and Harwood, 2007).

Increasing milk production of dairy cows during early-lactation is a good strategy to increase farm profits. Roche et al. (2013) reported that producing 1 extra kilogram of milk at the peak of lactation can result in more than 200 kg of milk throughout the whole lactation period. In our study, supplementing early-lactation cows (20 ± 5.0 DIM) with CSPF up to 0.6 kg/d for 90 d maintained a higher milk production until 202 d of the experiment. Previous studies reported that supplementing CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect from 16 to approximately 30 wk postpartum (Batistel et al., 2017; de Souza et al., 2017). A positive carryover effect was also observed in early-lactation dairy cows supplemented at 1.5% diet DM with FA blends containing different ratios of C16:0 and *cis*-9 C18:1 (de Souza et al., 2021). One of the possible reasons for the carryover effect is the increase in nutrient availability to the mammary gland, which stimulates secretory cell proliferation, promoting greater lactation persistency (Knight, 2000; Nørgaard et al., 2005). Also, we did not evaluate nutrient digestibility during the carryover period, so the digestibility effects observed during the treatment period may have persisted. It is noteworthy that we switched from a treatment period with grazing cows to a carryover period with a TMR diet. Possibly, this switching of feeding management influenced the carryover responses and we could have obtained different outcomes by using other strategies. The mechanisms underlying the carryover effects are still unclear and warrant further investigation.

CONCLUSIONS

Supplementing CSPF increased NDF and total fat digestibility as well as the yields of milk, milk fat, 3.5% FCM, ECM, and feed efficiency in early-lactation dairy cows grazing on tropical pastures. Although total fat digestibility had a quadratic response, most production measurements linearly increased during the treatment period, indicating that 0.6 kg/d CSPF was the best dose. Therefore, improvements in production might continue with higher doses. However, partially replacing a corn-based supplement with CSPF linearly decreased DMI, BW change, and BCS in early lactation. Including CSPF up to 0.6 kg/d for 90 d consistently increased milk yield, 3.5% FCM, and ECM and maintained a positive carryover effect into later lactation. Future CSPF and overall fat supplementation studies should more deeply investigate the mechanisms underlying the carryover effects.

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