J. Dairy Sci. 104:9752–9768
https://doi.org/10.3168/jds.2020-19936

Effects of calcium salts of palm fatty acids on nutrient digestibility and production responses of lactating dairy cows: A meta-analysis and meta-regression

J. M. dos Santos Neto,[1] J. de Souza,[2] and A. L. Lock[1]*
[1]Department of Animal Science, Michigan State University, East Lansing 48824
[2]Perdue AgriBusiness, Salisbury, MD 21804

ABSTRACT

Our primary objective was to perform a meta-analysis and meta-regression to evaluate the effects of diets supplemented with calcium salts of palm fatty acids (CSPF) compared with nonfat supplemented control diets (CON) on nutrient digestibility and production responses of lactating dairy cows. Our secondary objective was to perform a meta-analysis to evaluate whether experimental design affects production responses to supplemental CSPF. The data set was formed from 33 peer-reviewed publications with CSPF supplemented at $\leq$3% diet dry matter. We analyzed the interaction between experimental design (continuous vs. change-over) and treatments (CON vs. CSPF) to evaluate whether experimental design affects responses to CSPF (Meta.1). Regardless of experimental design, we evaluated the effects of CSPF compared with CON on nutrient digestibility and production responses of lactating dairy cows by meta-analysis (Meta.2) and meta-regression (Meta.3) approaches. In Meta.1, there was no interaction between treatments and experimental design for any variable. In Meta.2, compared with CON, CSPF reduced dry matter intake [DMI, 0.56 $\pm$ 0.21 kg/d ($\pm$SE)] and milk protein content (0.05 $\pm$ 0.02 g/100 g), increased neutral detergent fiber (NDF) digestibility (1.60 $\pm$ 0.57 percentage units), the yields of milk (1.53 $\pm$ 0.56 kg/d), milk fat (0.04 $\pm$ 0.02 kg/d), and 3.5% fat corrected milk (FCM, 1.28 $\pm$ 0.60 kg/d), and improved feed efficiency [energy corrected milk (ECM)/DMI, 0.08 kg/kg $\pm$ 0.03]. There was no effect of treatment for milk protein yield, milk fat content, body weight, body weight change, or body condition score. Compared with CON, CSPF reduced the yield of de novo milk fatty acids (FA) and increased the yields of mixed and preformed milk FA. In Meta.3, we observed that each 1-percentage-unit increase of CSPF in diet dry matter reduced DMI, increased NDF digestibility, tended to increase FA digestibility, increased the yields of milk, milk fat, and 3.5% FCM, reduced the content of milk protein, reduced the yield of de novo milk FA, and increased the yields of mixed and preformed milk FA. In conclusion, our results indicate no reason for the restrictive use of change-over designs in CSPF supplementation studies or meta-analysis. Feeding CSPF increased NDF digestibility, tended to increase FA digestibility, and increased the yields of milk, milk fat, and 3.5% FCM. Additionally, CSPF increased milk fat yield by increasing the yields of mixed and preformed milk FA.

Key words: experimental design, meta-analysis, fatty acid supplementation, dairy cow

INTRODUCTION

The addition of supplemental fatty acid (**FA**) sources to diets is a common practice in dairy nutrition to increase dietary energy content, feed efficiency, and the yields of milk and milk components (Rabiee et al., 2012). In a recent review, Palmquist and Jenkins (2017) discussed the historical progress of fat feeding in dairy cows, starting from the use of fats naturally present in feeds, to commercially available fat supplements designed to have minimal effects on rumen fermentation. Calcium salts of FA are one of the most common rumen-inert FA supplements used to minimize the negative effect of unsaturated FA on ruminal fermentation (Palmquist, 1991).

Initially, research with sheep observed that dietary inclusion of cations could alleviate the negative effects of unsaturated FA on DM and cellulose digestibility (Grainger et al., 1961; Davison and Woods, 1963). The ionic bonds between calcium and other metals with FA are affected by pH, in which release of calcium ions is directly correlated with changes in pH (Sukhija and Palmquist, 1990). In addition, studies on ruminal li-

Received November 20, 2020.
Accepted April 27, 2021.
*Corresponding author: allock@msu.edu

polysis and biohydrogenation indicated that only free carboxyl groups of FA would have the potential to be harmful to rumen microorganisms (Hawke and Robertson, 1964; Hawke and Silcock, 1969). Calcium salts of FA were developed to be insoluble at rumen pH and dissociate at the low pH in the abomasum (Jenkins and Palmquist, 1984; Schneider et al., 1988; Loften and Cornelius, 2004).

However, the ruminal dissociation pattern of calcium salts of FA is dependent on FA profile. Sukhija and Palmquist (1990) observed that calcium salts of highly unsaturated FA had high rumen dissociation, whereas calcium salts of palm fatty acids (**CSPF**) were satisfactorily stable to a pH of 5.5. The CSPF typically contain palmitic (C16:0; ~45%) and oleic (*cis*-9 C18:1; ~35%) acids (Loften and Cornelius, 2004; de Souza et al., 2019). A previous meta-analysis by Rabiee et al. (2012) evaluated the effects of fat supplementation on lactating dairy cows and demonstrated that compared with nonfat supplemented control diets, CSPF decreased DMI and increased milk yield. However, in the abovementioned study there was no limit on supplemental fat inclusion, so some of the CSPF inclusion levels were higher than those commonly used under most current farm conditions. In addition, Rabiee et al. (2012) focused on the effect of supplementation on production performance and did not determine the effect of CSPF on nutrient digestibility or milk FA composition.

Furthermore, change-over studies were excluded from the meta-analysis by Rabiee et al. (2012), because of concerns related to potential carryover effects from one period to another. Due to this, several meta-analyses have used only studies with continuous designs (Duffield et al., 2008a,b; Rodney et al., 2015). Lean et al. (2009) proposed that Latin square designs should be avoided in meta-analyses, by citing an oil and monensin study in lactating dairy cows (Cant et al., 1997), in which 2 cows previously fed fish oil had detectable docosahexaenoic acid in milk fat even after switching to another treatment. It is interesting to note that if this criterion of exclusion can be applied to meta-analyses, this would also invalidate the use of crossover and Latin square designs in all animal nutrition trials. Nonetheless, meta-analyses by Huhtanen and Hetta (2012) and Zanton (2019) provide evidence that change-over designs are as accurate as continuous designs in estimating production responses of lactating dairy cows receiving diets with different protein concentrations.

In fact, it does not seem reasonable to invalidate all research information produced from change-over designs based solely on individual observations from some animals. Change-over designs are built on the assumption of little or no carryover effect (Hu et al., 2017), in a way to ensure fair hypothesis-testing. Thereby, the most appropriate assessment consists of verifying whether responses to fat supplementation obtained in change-over designs follow the same pattern as responses obtained in continuous designs.

Therefore, our primary objective was to perform a meta-analysis and meta-regression to evaluate the effects of CSPF supplementation on nutrient digestibility and production responses of lactating dairy cows. Our second objective was to evaluate whether experimental design affects production responses to CSPF supplementation.

MATERIALS AND METHODS

Study Selection and Data Set

To perform our study, we searched for peer-reviewed papers that at least contained a comparison between a nonfat supplemented control diet (**CON**) with a diet supplemented with CSPF. Supplementation of CSPF had to be ≤3% DM in the diet of lactating dairy cows as the unique fat supplement (grazing dairy cows were not included).

Papers were searched for in electronic databases (Scirus, CAB, Cambridge University Press, Elsevier, Google Scholar, PubMed, ScienceDirect, and Springer) and in the search engines of *Animal Feed Science and Technology*, *Animal Production Science*, *Animal*, *Brazilian Journal of Animal Science, Journal of Animal Physiology and Animal Nutrition*, *Journal of Animal Science*, *Journal of Dairy Research*, *Journal of Dairy Science*, *Journal of the Science of Food and Agriculture*, *Livestock Science*, and *The Professional Animal Scientist* (*Applied Animal Science*). We used the following key words: calcium salts, calcium salts of fatty acids, calcium salts of palm fatty acids, dairy cow, digestibility, fat, fat supplementation, fatty acid, lipid, milk composition, milk yield, oleic, palm, palmitic, protected fats, and stearic. We also reviewed citations from previous reviews related to CSPF supplementation (Firkins and Eastridge, 1994; Allen, 2000; Loften and Cornelius, 2004; Onetti and Grummer, 2004; Rabiee et al., 2012; Loften et al., 2014; Boerman et al., 2015; Dórea and Armentano, 2017; Dórea et al., 2017; Palmquist and Jenkins, 2017; Weld and Armentano, 2017). Our final data set consisted of 33 publications published between 1988 and 2019 (Appendix; Supplemental Table S1; https://doi.org/10.5281/zenodo.4728005). We developed a PRISMA diagram according to Page et al. (2021) to describe the data collection process, which resulted in our final data set (Supplemental Figure S1 https://doi.org/10.5281/zenodo.4728005).

Data and Calculations

We performed 2 meta-analyses and one meta-regression. In the first meta-analysis (Meta.1), we evaluated whether experimental design affects nutrient digestibility and production responses to CSPF supplementation. Experimental designs were classified as either continuous (complete randomized and randomized as block) or change-over (crossover and Latin square), and treatments were CON and CSPF. We tested the interaction between experimental design and treatment. A description of the experimental designs used in the papers in our data set is in Supplemental Table S1. In the second meta-analysis (Meta.2), regardless of experimental design, we evaluated the effect of CSPF compared with CON on nutrient digestibility and production responses of lactating dairy cows.

Regardless of experimental design, we also performed a meta-regression to evaluate the dose response of CSPF on nutrient digestibility and production responses of lactating dairy cows (Meta.3). Meta.3 was performed according to Weld and Armentano (2017), wherein we calculated the difference between CSPF means minus CON means, which resulted in one observation per CSPF-CON pair (Δ). The Δ values were calculated for the concentration of supplemental FA (ΔFA, diet DM) and for the variables (Δvariables). Then, we used Δvariables as the dependent variable (Y) and ΔFA (diet DM) as the independent variable (X).

Across some studies, values were incomplete or not uniformly reported, which required the following calculations. Total CP was converted to milk CP (Schauff and Clark, 1989, 1992; Schauff et al., 1992; DeFrain et al., 2005), considering that nonprotein nitrogen represents approximately 6% of milk CP (NRC, 2001; Hu et al., 2017). As many of the experiments estimated milk protein from CP as 94% of the value, we assumed that using this formula would not affect our results. For studies that reported EE instead of FA, total FA content of the diet (Schneider et al., 1988; Salfer et al., 1994; Simas et al., 1995; Rodriguez et al., 1997; Moallem et al., 2010) was estimated as FA = EE − 1 (NRC, 2001). Yields of 3.5% FCM and ECM were calculated using the yields of milk and milk components as follows:

$$3.5\%\ \text{FCM} = [(0.4324 \times \text{kg of milk}) + (16.216 \times \text{kg of milk fat})],$$

$$\text{ECM} = [(0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of milk fat}) + (7.20 \times \text{kg of milk protein})].$$

We also calculated feed efficiency by dividing ECM by DMI.

Yields of individual FA (g/d) in milk fat were calculated using milk fat yield and individual FA concentrations, correcting milk fat yield for glycerol content and other milk lipid classes (Piantoni et al., 2013). The summation of milk FA concentrations and yields by source (de novo [Σ < C16], mixed [Σ C16 + C16:1], preformed [Σ >C16]) was also calculated. We report total values for C18:1, C18:2 and C18:3 rather than individual isomers.

Energy output (Mcal/d) for milk and maintenance were calculated according to NRC (2001): Milk energy output (Mcal/d) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (%)], when lactose % was not reported, we used 4.85% (NRC, 2001); and maintenance energy output (Mcal/d) = kg $BW^{0.75}$ (kg) × 0.08.

Descriptive statistics for FA composition of CSPF supplements, nutrient composition of dietary treatments, variables used to evaluate experimental designs (Meta.1), and variables used to evaluate the effects of CSPF supplementation (Meta.2 and Meta.3) are shown in Tables 1 and 2 and in Supplemental Tables S2 and S3 (https://doi.org/10.5281/zenodo.4728005), respectively. Descriptive statistics for individual FA used to evaluate the effects of CSPF (Meta.2 and Meta.3) are shown in Supplemental Table S4 (https://doi.org/10.5281/zenodo.4728005). In Meta.1, we did not evaluate FA digestibility due to limited observations with continuous designs, or BW change and BCS due to limited observations with change-over designs.

Publication Bias

Publication bias is mainly associated with the tendency for studies reporting nonsignificant results less likely to be published. The funnel plot is a simple technique to help assess possible publication bias, where the effect estimate is plotted against some measure of precision (Sterne and Harbord, 2004; Peters et al., 2008; Rabiee et al., 2012). An asymmetrical appearance of the funnel plot with evident gaps in the graph indicates publication bias (Sterne and Harbord, 2004; Sterne et al., 2011; Rabiee et al., 2012). Peters et al. (2008) proposed the use of contour-enhanced funnel plots, where contour lines indicate areas of statistical significance and nonsignificance. Using this approach, it is easy to visualize if the gaps are in nonsignificant areas, which may indicate publication bias (Peters et al., 2008). We tested for publication bias using both standard funnel plots (Sterne and Harbord, 2004; Ra-

Table 1. Descriptive statistics of the calcium salts of palm fatty acid supplements (CSPF) in the data set

Item	n[1]	CSPF				
		Mean	Median	Min	Max	SD
CSPF added in the diet, %	60	2.20	2.40	0.78	3.00	0.62
FA profile, g/100 g						
C16:0	6	46.0	45.2	41.8	51.5	3.40
C18:0	6	3.84	4.25	0.69	5.00	1.62
C18:1	6	37.9	39.1	33.3	40.2	2.87
C18:2	6	8.35	8.39	7.40	9.50	0.88

[1]Number of treatment means with supplemental CSPF from the 33 experiments, and the treatment means that reported the fatty acid (FA) profile of the CSPF fed.

biee et al., 2012) and contour-enhanced funnel plots (Sterne and Egger, 2001; Peters et al., 2008). The funnel plots were generated for some of the key response variables that potentially could lead to some degree of publication bias: milk yield, milk fat yield, milk protein yield, milk fat content, milk protein content, and NDF digestibility (Supplemental Figure S2; https://doi.org/10.5281/zenodo.4728005).

Weighting of Observations

The mean of dependent variables directly extracted from selected peer-reviewed papers were weighted by the inverse of the squares of their standard errors as recommended by St-Pierre (2001). This allowed us to maintain the expressions of dispersion in the original scale of the measurements (St-Pierre, 2001, 2007). For dependent variables that were calculated, we used the number of experimental units rather than standard error to define the weighting factors (St-Pierre, 2001, 2007).

Statistical Analysis

We generated funnel plots to evaluate publication bias using the metafor package (Viechtbauer, 2010) of R Software (version 1.3.1093; https://cran.r-project.org/web/packages/metafor). All other statistical analyses were performed using SAS Software (version 9.4, SAS Institute).

In Meta.1, we used the following model:

$$Yijk = \mu + T_i + E_j + B_k + T_i \times E_j + e_{ijk},$$

where Y_{ijk} = dependent variable, μ = overall mean, T_i = fixed effect of treatments, E_j = fixed effect of experimental designs, B_k = random effect of study, $T_i \times E_j$ = fixed effect of interaction between treatments and experimental design, and e_{ijk} = residual error.

In Meta.2, we used the following model:

$$Y_{ik} = \mu + T_i + B_k + e_{ik},$$

where Y_{ik} = dependent variable, μ = overall mean, T_i = fixed effect of treatments, B_k = random effect of study, and e_{ik} = residual error. In addition, we also performed a separate analysis, where DMI was used as a covariate to determine effects of CSPF on nutrient digestibility (Cov = effect of covariate). As DMI can affect nutrient digestibility, our aim was to isolate this factor to better understand the effects of CSPF on nutrient digestibility (especially NDF digestibility).

In Meta.3, the intercepts were forced through zero and only the slope values were estimated, which represents no fat addition or CON (forced zero intercept). This assumption was also used by Weld and Armentano (2017). Therefore, we used the following model for linear terms:

$$Y_{ik} = D_iC_i + B_k + e_{ik},$$

Table 2. Descriptive statistics of the nutrient composition of nonfat supplemented control diets (CON) and diets supplemented with calcium salts of palm fatty acids (CSPF) in the data set

Nutrient composition, % of DM	n[1]	CON					n[1]	CSPF				
		Mean	Median	Min	Max	SD		Mean	Median	Min	Max	SD
NDF	39	33.1	32.2	25.2	47.0	5.18	43	32.5	32.3	24.8	46.0	4.68
CP	43	18.0	18.0	15.2	22.0	1.97	47	17.9	17.5	15.6	21.4	1.70
FA[2]	38	3.45	3.61	1.40	6.76	1.36	42	5.08	4.90	2.40	8.63	1.23

[1]Number of treatment means from the 33 experiments that reported the nutrient composition of CON and CSPF.

[2]FA = fatty acids.

where Y_{ik} = dependent variable, D_iC_i = slope of the Δtreatments due to CSPF in the diet (%DM), B_k = random effect of study, and e_{ik} = residual error.

All data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute). WEIGHT statement was used to provide a weight for each observation in the input data set. We assessed the assumptions of the meta-analytic model according to St-Pierre (2007). Homoscedasticity was assessed by scatterplots of the residuals against the predicted values. The equations for Meta.3 were adjusted to a linear model with the intercept forced to zero by the NOINT option. In this paper, what previously would have been termed RMSE (the square root of the estimated residual variance) is reported as $\hat{\sigma}_e$ (estimated σ for error). We also estimated the square root of the estimated variance due to study as $\hat{\sigma}_s$ (estimated σ for study; Boerman et al., 2015).

Differences between means in Meta.1 and Meta.2 were determined using the P-DIFF option of the LSMEANS statement. In Meta.3, the slopes from linear models were tested to determine if they were different from zero. Significant differences were declared at $P \leq 0.05$, and tendencies at $0.05 < P \leq 0.10$. Results from Meta.1 are reported in tables as the mean difference between change-over and continuous designs. Results from Meta.2 are reported in tables as the mean difference between CON and CSPF. Results from Meta.3 are reported as the effect of 1-percentage-unit increases of CSPF in diet DM.

RESULTS

Publication Bias

We did not identify any marked asymmetry for the key responses analyzed using standard funnel plots (Supplemental Figure S2). In addition, we did not observe important gaps in nonsignificant areas of the contour-enhanced funnel plots, indicating little evidence of publication bias (Supplemental Figure S2).

Meta.1: Effect of Experimental Design on DMI, Nutrient Digestibility, and Production Responses of Lactating Dairy Cows Supplemented with CSPF

There was no interaction or tendency for interactions between treatment and experimental design for any production variable ($P \geq 0.27$, Tables 3, 4). Overall, compared with continuous, change-over design increased the content of milk protein ($P = 0.02$, Table 3) and tended to increase the yield of mixed milk FA ($P = 0.06$, Table 4). We did not observe an effect of experimental design on any other production variable or source of milk FA ($P \geq 0.32$, Tables 3, 4).

The effect of experimental design and the interactions between treatment and experimental design on individual milk FA are not reported in the tables, but they are briefly described here. We observed no interactions or tendencies for interactions between treatment and experimental design for individual milk FA. Compared with continuous, change-over design increased the yields of C14:0 (37.7 ± 16.1 g/d, $P = 0.03$) and C16:0 (150 ± 34.3 g/d, $P < 0.01$), decreased the concentrations of C4:0 (0.97 ± 0.41 g/100 g, $P = 0.03$) and C18:1 (6.99 ± 1.31 g/100 g, $P < 0.01$), increased the concentration of C18:3 (0.54 ± 0.18 g/100 g, $P = 0.02$), and tended to decrease the concentration of C6:0 ($P = 0.06$). We did not observe effects of experimental design on any other individual milk FA ($P \geq 0.12$).

Meta.2: Effect of CSPF Supplementation on DMI, Nutrient Digestibility, and Production Responses of Lactating Dairy Cows

Compared with CON, CSPF reduced DMI ($P = 0.01$), increased NDF digestibility ($P = 0.01$), and had no effect on DM ($P = 0.85$), CP ($P = 0.32$), or FA digestibility ($P = 0.12$, Table 5). Because CSPF decreased DMI, and this is an important factor affecting nutrient digestibility, we also tested DMI as a covariate to determine the effects of CSPF on nutrient digestibility. As a covariate, DMI tended to be significant for NDF digestibility [β coefficient = −0.78 ± 0.39 (± SE), $P = 0.06$], but it had no effect on the digestibilities of DM ($P = 0.26$), CP ($P = 0.46$), or FA ($P = 0.59$). When we kept DMI as a covariate in the model, CSPF tended to increase NDF digestibility compared with CON by 1.09 ± 0.61 percentage units ($P = 0.08$) and had no effect on the digestibilities of DM ($P = 0.91$), CP ($P = 0.61$), and FA ($P = 0.11$, data not shown in the tables).

Production Responses

Compared with CON, CSPF increased the yields of milk ($P < 0.01$), milk fat, 3.5% FCM ($P = 0.04$), and energy output to milk ($P = 0.01$), reduced the content of milk protein ($P = 0.02$, Table 5), tended to increase ECM yield ($P = 0.07$), and improved feed efficiency (ECM/DMI, $P = 0.01$). There was no effect of treatment on milk protein yield ($P = 0.94$), milk lactose yield ($P = 0.77$), milk fat content ($P = 0.43$), milk lactose content ($P = 0.34$), BW ($P = 0.36$), BW change ($P = 0.25$), BCS ($P = 0.15$), or energy output for maintenance ($P = 0.45$, Table 5).

Table 3. Meta.1: Effect of experimental design and its interaction with supplementation of calcium salts of palm fatty acids on DMI, nutrient digestibility, production responses, and energy output of cows

Item	Mean difference			Variance[3]		*P*-value[4]		
	Estimate[1]	SE	n[2]	$\hat{\sigma}_s$	$\hat{\sigma}_e$	Trt	Design	Trt × Design
DMI, kg/d	1.71	1.14	30 (102)	2.68	1.09	0.03	0.14	0.68
Nutrient digestibility, %								
DM	0.69	1.47	12 (36)	2.25	1.40	0.99	0.64	0.78
CP	0.85	3.58	9 (26)	5.01	2.32	0.34	0.82	0.67
NDF	−2.11	5.24	12 (40)	8.89	1.41	0.01	0.69	0.27
Yield, kg/d								
Milk	−0.30	2.07	31 (100)	4.56	2.85	0.02	0.88	0.90
3.5% FCM[5]	0.02	0.25	26 (88)	5.22	1.33	0.04	0.63	0.85
ECM[6]	0.02	0.25	25 (84)	5.55	1.34	0.07	0.90	0.89
Fat	0.07	0.08	27 (98)	0.17	0.10	0.02	0.42	0.26
Protein	−0.03	0.05	26 (94)	0.00	0.10	0.94	0.54	0.96
Concentration, g/100 g								
Fat	0.15	0.16	31 (107)	0.30	0.22	0.43	0.35	0.59
Protein	0.14	0.06	29 (101)	0.10	0.10	0.01	0.02	0.20
BW, kg/d	−7.54	34.3	14 (50)	43.7	31.2	0.36	0.83	0.83
Energy output,[7] Mcal/d								
Milk	1.51	1.50	28 (91)	3.07	1.35	0.13	0.32	0.75
Maintenance	−0.03	0.33	14 (50)	0.46	0.22	0.52	0.92	0.67

[1]Difference between experimental designs (Change-over – Continuous).

[2]Number of studies (overall number of treatment means); more information in Supplemental Table S2 (https://doi.org/10.5281/zenodo.4728005).

[3] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[4]Trt = treatments (Control vs. Calcium salts of palm fatty acids); Design = experimental designs (Change-over vs. Continuous); and Trt × Design = interaction between treatments and experimental designs.

[5]3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)] (NRC, 2001).

[6]ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)] (NRC, 2001).

[7]Energy output to milk (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)]; Energy output to maintenance (Mcal/d) = $BW^{0.75}$ (kg) × 0.08 (NRC, 2001).

Milk Fatty Acid Yields and Concentrations

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 from plasma extraction. Compared with CON, CSPF decreased the

Table 4. Meta.1: Effect of experimental design and its interaction with supplementation of calcium salts of palm fatty acids on sources of milk fatty acids of cows

Item[1]	Mean difference			Variance[4]		*P*-value[5]		
	Estimate[2]	SE	n[3]	$\hat{\sigma}_s$	$\hat{\sigma}_e$	Trt	Design	Trt × Design
Summation by source, g/d								
De novo	38.2	49.3	7 (28)	2.00	0.45	<0.01	0.45	0.46
Mixed	107	54.6	9 (32)	2.46	0.48	0.03	0.06	0.41
Preformed	30.2	9.00	8 (30)	3.81	0.74	<0.01	0.74	0.32
Summation by source, g/100 g								
De novo	−1.41	3.23	7 (28)	3.99	1.64	<0.01	0.67	0.61
Mixed	1.10	3.86	9 (32)	5.62	0.50	0.06	0.79	0.86
Preformed	−4.06	5.43	8 (30)	7.46	1.01	<0.01	0.47	0.14

[1]De novo fatty acids originate from mammary de novo synthesis (<16 carbons), preformed fatty acids originate from extraction from plasma (>16 carbons), and mixed fatty acids originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Supplemental Table S5 (https://doi.org/10.5281/zenodo.4728005).

[2]Difference between experimental designs (Change-over – Continuous).

[3]Number of studies (overall number of treatment means). More information is in Supplemental Table S2 (https://doi.org/10.5281/zenodo.4728005).

[4] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[5]Trt = treatments (control vs. calcium salts of palm fatty acids); Design = experimental designs (Change-over vs. Continuous); and Trt × Design = interaction between treatments and experimental designs.

Table 5. Meta.2: Dry matter intake, nutrient digestibility, production responses, and energy output of cows fed nonfat supplemented control diets (CON) or diets supplemented with calcium salts of palm fatty acids (CSPF)

Item	Mean difference			Variance[3]		
	Estimate[1]	SE	n[2]	$\hat{\sigma}_s$	$\hat{\sigma}_e$	P-value[4]
DMI, kg/d	−0.56	0.21	30 (102)	2.72	1.09	0.01
Nutrient digestibility, %						
DM	0.08	0.46	12 (36)	2.16	1.38	0.85
CP	0.91	0.89	9 (26)	4.55	2.28	0.32
NDF	1.60	0.57	12 (40)	8.54	1.43	0.01
FA[5]	2.68	1.64	9 (26)	8.92	4.17	0.12
Yield kg/d						
Milk	1.53	0.56	31 (100)	4.48	2.83	<0.01
3.5% FCM[6]	1.28	0.60	26 (88)	5.12	1.32	0.04
ECM[7]	1.12	0.60	25 (84)	5.43	1.32	0.07
Fat	0.04	0.02	27 (98)	0.17	0.10	0.04
Protein	0.00	0.02	26 (94)	0.10	0.10	0.94
Lactose	0.02	0.09	7 (23)	0.26	0.10	0.77
ECM/DMI, kg/kg	0.08	0.03	24 (80)	0.20	0.06	0.01
Concentration, g/100 g						
Fat	0.03	0.04	31 (107)	0.30	0.22	0.43
Protein	−0.05	0.02	29 (101)	0.10	0.10	0.02
Lactose	0.02	0.02	9 (29)	0.10	0.00	0.34
BW, kg	−7.98	8.72	14 (50)	40.7	30.8	0.36
BW change, kg/d	−0.06	0.05	8 (22)	0.20	0.10	0.25
BCS	−0.10	0.06	6 (24)	0.00	0.14	0.15
Energy output,[8] Mcal/d						
Milk	0.91	0.32	28 (91)	3.07	1.35	0.01
Maintenance	−0.05	0.06	14 (50)	0.42	0.22	0.45

[1]Difference between treatments (CSPF – CON).

[2]Number of studies (overall number of treatment means). More information in Supplemental Table S3 (https://doi.org/10.5281/zenodo.4728005).

[3] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[4]P-values associated with the effects of CON vs. CSPF.

[5]FA = fatty acids.

[6]3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)] (NRC, 2001).

[7]ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)] (NRC, 2001).

[8]Energy output to milk (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)]; Energy output to maintenance (Mcal/d) = $BW^{0.75}$ (kg) × 0.08 (NRC, 2001).

yield of de novo milk FA ($P < 0.01$, Table 6), which was driven by a reduction in the yield of C6:0 ($P = 0.02$), C8:0, C10:0, C12:0, C14:0, and C14:1 (all $P < 0.01$, Supplemental Table S5; https://doi.org/10.5281/zenodo.4728005). There was no effect of treatment on the yield of C4:0 ($P = 0.12$, Supplemental Table S5). Compared with CON, CSPF increased the yield of mixed milk FA ($P = 0.01$, Table 6), due to an increase in C16:0 ($P = 0.02$) and no effect on C16:1 ($P = 0.44$, Supplemental Table S5). We observed that CSPF increased the yield of preformed ($P < 0.01$, Table 6) compared with CON, primarily due to an increase in the yield of total C18:1 ($P < 0.01$, Supplemental Table S5). Overall, we observed a similar pattern of results for the effects of treatments on a concentration basis (g/100 g) compared with a yield basis (g/d; Table 6, Supplemental Table S5). However, CSPF increased C4:0 content of milk fat ($P = 0.03$, Supplemental Table S5).

Meta.3: Dose Response of CSPF on DMI, Nutrient Digestibility, and Production Responses of Lactating Dairy Cows

In our data set, the inclusion of CSPF ranged from 0.78 to 3.00% diet DM, with an average of 2.20% diet DM (Table 1). Initially, we analyzed linear and quadratic relationships between ΔFA and Δvariables. We did not observe any quadratic effects of increasing CSPF supplementation, and the intercepts from the linear models were not found to be significant or to have tendencies ($P = 0.95$, data not shown). Therefore, we used a linear model by forcing intercepts through zero, which represents no CSPF supplementation or CON (Weld and Armentano, 2017). We describe the effects of increasing CSPF supplementation by reporting response values for each 1-percentage-unit increase of CSPF in diet DM.

Table 6. Meta.2: Milk fatty acid yields and concentrations by source of cows fed nonfat supplemented control diets (CON) or diets supplemented with calcium salts of palm fatty acids (CSPF)

Item[1]	Mean difference		n[3]	Variance[4]		*P*-value[5]
	Estimate[2]	SE		$\hat{\sigma}_s$	$\hat{\sigma}_e$	
Summation by source, g/d						
De Novo	−41.1	5.34	7 (28)	1.95	0.45	<0.01
Mixed	13.4	5.31	9 (32)	2.99	0.47	0.01
Preformed	68.0	15.4	8 (30)	3.59	0.74	<0.01
Summation by source, g/100 g						
De Novo	−3.99	0.61	7 (28)	3.69	1.61	<0.01
Mixed	1.28	0.55	9 (32)	5.30	0.50	0.05
Preformed	4.04	0.80	8 (30)	7.24	1.00	<0.01

[1]De novo fatty acids originate from mammary de novo synthesis (<16 carbons), preformed fatty acids originate from extraction from plasma (>16 carbons), and mixed fatty acids originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Supplemental Table S5 (https://doi.org/10.5281/zenodo.4728005).

[2]Difference between treatments (CSPF – CON).

[3]Number of studies (overall number of treatment means). More information in Supplemental Table S3 (https://doi.org/10.5281/zenodo.4728005).

[4]$\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[5]*P*-values associated with the effects of CON vs. CSPF.

DMI and Nutrient Digestibility

We observed that each 1-percentage-unit increase of CSPF in diet DM reduced DMI (0.37 kg/d, $P < 0.01$), had no effect on the digestibilities of DM ($P = 0.86$) and CP ($P = 0.20$), increased NDF digestibility (1.15 percentage units, $P = 0.03$), and tended to increase FA digestibility (1.61 percentage units, $P = 0.09$, Table 7).

Production Responses

We observed that each 1-percentage-unit increase of CSPF in diet DM increased the yields of milk (0.54 kg/d, $P = 0.01$), 3.5% FCM (0.46 kg/d, $P = 0.02$), ECM (0.39 kg/d, $P = 0.03$) and milk fat (25.6 g/d, $P = 0.05$) and had no effect on milk protein yield ($P = 0.90$, Table 7). Each 1-percentage-unit increase of CSPF in diet DM had no effect on milk fat concentration ($P = 0.90$), reduced milk protein concentration (0.03 g/100 g, $P = 0.05$), and increased energy output to milk (0.33 Mcal/d, $P = 0.03$, Table 7). Body weight ($P = 0.20$), BW change ($P = 0.90$), BCS ($P = 0.68$), and energy output to maintenance ($P = 0.95$, Table 7) were not affected by increasing CSPF.

Milk Fatty Acid Yields and Concentrations

We observed that each 1-percentage-unit increase of CSPF in diet DM decreased the yield of de novo (21.1 g/d, $P < 0.01$) and increased the yields of mixed (10.2 g/d, $P = 0.03$) and preformed milk FA (26.9 g/d, $P < 0.01$, Table 8). For the yield of individual milk FA, each 1-percentage-unit increase of CSPF in diet DM had no effect on C4:0 ($P = 0.36$), C16:1 ($P = 0.18$), C18:0 ($P = 0.18$), C18:2 ($P = 0.14$) and C18:3 ($P = 0.42$), decreased C6:0 (1.00 g/d, $P = 0.03$), C8:0 (1.27 g/d), C10:0 (3.81 g/d), C12:0 (4.85 g/d), C14:0 (10.1 g/d, $P < 0.01$) and C14:1 (1.32 g/d, $P = 0.03$), and increased C16:0 (10.6 g/d, $P = 0.03$) and C18:1 (24.2 g/d, $P = 0.01$, Supplemental Table S6; https://doi.org/10.5281/zenodo.4728005).

On a concentration basis, each 1-percentage-unit increase of CSPF in diet DM decreased the concentration of de novo (2.05 g/100 g, $P < 0.01$), and increased the concentrations of mixed (0.51 g/100 g, $P = 0.03$) and preformed milk FA (1.85 g/100 g, $P < 0.01$, Table 8). On a concentration basis, CSPF had no effect on C4:0 ($P = 0.55$), C16:1 ($P = 0.45$), C18:0 ($P = 0.76$), C18:2 ($P = 0.68$) and C18:3 ($P = 0.44$), decreased C6:0 (0.10 g/100 g, $P = 0.01$), C8:0 (0.11 g/100 g), C10:0 (0.33 g/100 g), C12:0 (0.42 g/100 g), C14:0 (0.95 g/100 g) and C14:1 (0.09 g/100 g, $P < 0.01$), and increased C16:0 (0.56 g/100 g, $P = 0.02$) and C18:1 (1.88 g/100 g, $P < 0.01$, Supplemental Table S6).

DISCUSSION

Previous systematic reviews have shed light on the effects of dietary supplementation of CSPF to lactating dairy cows (Allen, 2000; Onetti and Grummer, 2004; Rabiee et al., 2012; Weld and Armentano, 2017). However, in these studies, there was no limit on CSPF inclusion, thus, some of the reported doses were higher than those commonly used on farms under most typical feeding situations. Based on our interactions with field nutritionists and farmers, most commercial dairy farms

Table 7. Meta.3: DMI, nutrient digestibility, production responses, and energy output of cows for each 1-percentage-unit increase of calcium salts of palm fatty acids (CSPF) in diet DM

Item	Inclusion/1% DM[1]		n[2]	Variance[3]		*P*-value[4]
	CSPF	SEM		$\hat{\sigma}_s$	$\hat{\sigma}_e$	CSPF/1%DM
DMI, kg/d	−0.37	0.08	30 (54)	0.00	0.86	<0.01
Nutrient digestibility, %						
DM	0.05	0.27	12 (18)	0.99	1.33	0.86
CP	0.54	0.29	9 (13)	0.00	1.66	0.20
NDF	1.15	0.29	12 (18)	0.25	1.94	0.03
FA[5]	1.61	0.88	9 (13)	5.03	1.95	0.09
Yield kg/d						
Milk	0.54	0.18	31 (53)	1.22	0.57	0.01
3.5% FCM[6]	0.46	0.17	26 (47)	0.86	0.44	0.02
ECM[7]	0.39	0.16	25 (45)	0.84	0.65	0.03
Fat, g/d	25.6	12.2	27 (52)	2.23	1.27	0.05
Protein, g/d	1.11	8.43	26 (50)	1.41	1.39	0.90
Concentration, g/100 g						
Fat	0.00	0.02	31 (55)	0.08	0.12	0.90
Protein	−0.03	0.01	29 (52)	0.06	0.08	0.04
BW, kg	−3.08	1.96	14 (27)	5.47	11.20	0.20
BW change, kg/d	−0.01	0.03	8 (13)	0.00	0.17	0.90
BCS	−0.02	0.06	6 (13)	0.08	0.20	0.68
Energy output,[8] Mcal/d						
Milk	0.33	0.13	28 (47)	0.89	0.28	0.03
Maintenance	0.00	0.03	14 (27)	0.09	0.16	0.95

[1]Effect of 1-percentage-unit increase of CSPF in diet DM.

[2]Number of studies (the resulting number of observations obtained from the difference between CSPF diet means minus control diet [CON] means used in the meta-regression). More information in Supplemental Table S3 (https://doi.org/10.5281/zenodo.4728005).

[3] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[4]*P*-values were used to test the linear effect of the supplementation of CSPF per 1-percentage-unit increase in diet DM.

[5]Fatty acids.

[6]3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)] (NRC, 2001).

[7]ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)] (NRC, 2001).

[8]Energy output to milk (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)]; Energy output to maintenance (Mcal/d) = $BW^{0.75}$ (kg) × 0.08 (NRC, 2001).

Table 8. Meta.3: Milk fatty acid yields and concentrations by source of cows for each 1-percentage-unit increase of calcium salts of palm fatty acids (CSPF) in diet dry matter

Item[1]	Inclusion/1%DM[2]		n[3]	Variance[4]		*P*-value[5]
	CSPF	SEM		$\hat{\sigma}_s$	$\hat{\sigma}_e$	CSPF/1% DM
Summation by source, g/d						
De novo	−21.1	3.14	7 (15)	0.26	0.63	<0.01
Mixed	10.2	3.87	9 (17)	0.37	0.42	0.03
Preformed	26.9	5.28	8 (16)	0.83	0.28	<0.01
Summation by source, g/100 g						
De novo	−2.05	0.22	7 (15)	0.95	0.78	<0.01
Mixed	0.51	0.19	9 (17)	1.22	0.20	0.03
Preformed	1.85	0.21	8 (16)	0.87	0.48	<0.01

[1]De novo fatty acids originate from mammary de novo synthesis (<16 carbons), preformed fatty acids originate from extraction from plasma (>16 carbons), and mixed fatty acids originate from both sources (C16:0 plus *cis*-9 C16:1). Concentrations and yields of individual fatty acids are reported in Supplemental Table S6 (https://doi.org/10.5281/zenodo.4728005).

[2]Effect of 1-percentage-unit increase of CSPF in diet DM.

[3]Number of studies (the resulting number of observations obtained from the difference between CSPF diet means minus control diet [CON] means used in the meta-regression). More information in Supplemental Table S3 (https://doi.org/10.5281/zenodo.4728005).

[4] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

[5]*P*-values were used to test the linear effect of the supplementation of CSPF per 1-percentage-unit increase in diet DM.

include FA supplements at ≤3.0% of diet DM. Our goal was to perform a meta-analysis and a meta-regression to evaluate the effects of CSPF included at ≤3% in the diet DM on nutrient digestibility and production responses of lactating dairy cows. Additionally, concern has been raised as to the appropriateness of including change-over designs (e.g., crossover and Latin square) in meta-analyses (Lean et al., 2009). Therefore, we also performed a meta-analysis to evaluate whether experimental design affects responses to dietary supplementation of CSPF.

Overall, we did not identify any marked asymmetry for the key responses analyzed using standard funnel plots. Also, neither variable had important gaps in nonsignificant areas of the contour-enhanced funnel plots. If values do not appear to be missing in nonsignificant areas, there is little evidence of publication bias (Peters et al., 2008). It is important to emphasize that in our statistical models, we used study as a random effect. Random-effect models assume that the treatment effect is not the same across studies and adjusts not only for the within-study variance but also the between-study variance (St-Pierre, 2001; Fletcher, 2007; Baker et al., 2009). According to St-Pierre (2001), from a statistical standpoint, studies are blocks, and their effects must be considered random.

Concerns with change-over designs focus on the potential for carryover effects of previous treatments, and the short duration of experimental periods, which could influence outcomes (Lean et al., 2009; Zanton, 2019). Because of this, Rabiee et al. (2012) did not consider change-over designs in their meta-analysis to evaluate the effects of fat supplementation in dairy cattle diets. In our study, however, we observed no interactions between treatment and experimental design for any variable, clearly indicating that the responses obtained with CSPF supplementation in lactating dairy cows do not differ between continuous and change-over designs. Similarly, in a recent meta-analysis to evaluate the effects of supplementing saturated FA on milk production of dairy cows, Hu et al. (2017) observed that responses to supplemental saturated FA did not differ due to experimental design. Likewise, Zanton (2019) reported that most responses to differences in MP of dairy cow diets are consistent between trials randomized as block or Latin square designs, and their results go against the concern that change-over designs would not allow for detectable effects due to short experimental periods. This is further supported by the findings of Huhtanen and Hetta (2012). These authors also pointed out that change-over designs are built on the assumption of no carryover effects from one period to the next, and given their popularity in dairy nutrition studies, a wealth of research information would be wasted if such designs were excluded from a meta-analysis. The only effect of experimental design we observed was a reduction in milk protein content and a tendency for an increase in mixed milk FA yields for change-over studies compared with continuous designs. Such differences may be due to type of basal diets, dietary FA content, and stage of lactation across study types. This topic warrants further investigation. Nevertheless, in a meta-analysis, experimental design contributes to the random effect of the study, and the wide range of standard errors of treatments among studies due to the different statistical designs and number of experimental units can be easily balanced by the weighting of observations (St-Pierre, 2001, 2007). Therefore, based on our results and the discussion above, we do not see any reason for restricting the use of change-over designs in nutrition studies with CSPF supplementation or in the use of different study designs in meta-analyses.

To evaluate the effects of CSPF on nutrient digestibility and production responses of lactating dairy cows, treatment comparisons were obtained from both continuous and change-over designs. We observed that CSPF reduced DMI compared with CON. Reasons why some fat supplements reduce DMI have been extensively discussed by Allen (2000). The most recent findings indicate that hypophagic effects of CSPF are associated with the secretagogue actions of unsaturated FA on gut hormones and peptides related to satiety, such as cholecystokinin and glucagon-like peptide-1, respectively (Relling and Reynolds, 2007). Harvatine and Allen (2006) observed that increasing unsaturated FA in dairy cow diets linearly decreased DMI by 15%. Previous meta-analyses observed that CSPF decreased DMI compared with CON by 0.64 (Rabiee et al., 2012) and 0.97 kg/d (Onetti and Grummer, 2004). These authors, however, used studies in which the maximum CSPF inclusion was 6.10% diet DM. We observed that CSPF reduced DMI by 0.56 kg/d compared with CON, and we found a linear reduction of 0.37 kg/d for each 1-percentage-unit inclusion of CSPF in diet DM. Also, de Souza et al. (2018) reported that the inclusion of a supplemental FA blend of 45% C16:0 and 35% *cis*-9 C18:1 only decreased DMI of lactating dairy cows when included in a high FA basal diet with whole cottonseed due the high amount of unsaturated FA, given that the inclusion of the same supplemental FA blend had no effect on DMI when included in a low FA basal diet with soyhulls. Further research needs to be conducted to evaluate the interactions of CSPF with different dietary components.

The negative effects of supplemented fat on fiber digestibility of ruminants have been frequently cited (Devendra and Lewis, 1974; Jenkins and Palmquist, 1984). Many of these negative effects were related to the inclusion of pure oils and very high levels of fat feeding (Czerkawski, et al., 1966; Ikwuegbu and Sutton, 1982; Rodrigues et al., 2019), or calcium salts of soybean FA (Bettero et al., 2017; de Souza et al., 2017). Conversely, in a recent meta-analysis, Weld and Armentano (2017) observed that supplementation with CSPF had no effect on NDF digestibility. de Souza et al. (2018) fed a supplemental FA blend of 45% C16:0 and 35% *cis*-9 C18:1 at 1.77% of diet DM and observed an increase in NDF digestibility of 0.8 percentage units compared with a nonfat supplemented diet. Our results showed that CSPF increased NDF digestibility by 1.60 percentage units compared with CON. The increase in NDF digestibility with CSPF may be associated with a decrease in DMI, but this may not be the only reason, as the reduction in DMI did not alter the digestibility of DM itself. Indeed, when we tested DMI as a covariate, CSPF still tended to increase NDF digestibility compared with CON, but the difference was lower (1.09 percentage units). Overall, despite CSPF being satisfactorily stable at rumen pH, the release of calcium ions is directly correlated with the decrease in rumen pH, and even at an ideal pH range (6.0–6.5), some degree of dissociation occurs (Sukhija and Palmquist, 1990), so that some of the FA in CSPF are available in the rumen. Palmitic acid is the major FA in CSPF and is an important component in the biomembrane of fibrolytic bacteria (Mackie et al., 1991; Vlaeminck et al., 2006). Several studies have demonstrated that C16:0 supplementation increases NDF digestibility (e.g., de Souza et al., 2018; de Souza and Lock, 2019; Sears et al., 2020). Therefore, it is possible that CSPF increased NDF digestibility by a combination of both factors: the reduction of DMI and the effect of C16:0 on fibrolytic bacteria. In addition, CSPF have a slower rate of release of FA within the rumen and a lower extent of biohydrogenation compared with free oils and calcium salts of highly unsaturated FA (Sukhija and Palmquist, 1990; Wu et al., 1991). In fact, compared with calcium salts of soybean FA, CSPF increased NDF digestibility of grazing dairy cows by 8.8 percentage units (de Souza et al., 2017). The mechanisms underlying the increase in NDF digestibility by CSPF are still unclear and deserve further attention.

Increasing FA intake usually decreases total FA digestibility (Palmquist, 1991; Boerman et al., 2015). However, despite the overall higher intake of FA (1.10 vs. 0.77 kg/d) in CSPF supplemented diets, CSPF tended to increase FA digestibility by 1.61% units for each 1-percentage-unit increase in CSPF (diet DM) and did not decrease FA digestibility compared with CON. This is likely due to the unsaturated FA in CSPF (*cis*-9 C18:1 and *cis*-9, *cis*-12 C18:2), which have emulsifying properties (Freeman, 1969). It is important to note that due to rumen dissociation and subsequent biohydrogenation, it is likely that CSPF supplementation also increased rumen outflow of C18:0 (Jenkins and Bridges, 2007). Freeman (1969) studied the properties of FA in dispersions of emulsified lipid and bile salts and reported that *cis*-9 C18:1 positively affected the micellar solubility of C18:0. Similarly, de Souza et al. (2018) observed that a diet containing a supplemental FA blend of 45% C16:0 and 35% *cis*-9 C18:1 had similar FA digestibility to a control diet containing no supplemental FA, and increased the digestibilities of 16-carbon, 18-carbon, and total FA compared with a diet containing a supplemental FA blend of 40% C16:0 and 40% C18:0. Also, increasing *cis*-9 C18:1 from 10 to 30% in FA blends predominantly composed of C16:0 increased FA digestibility by 2.40 and 4.10 percentage units in fresh and mid-lactation cows, respectively (de Souza et al., 2019, 2021). These findings allow us to hypothesize that the FA profile reaching the duodenum may affect FA digestibility more than the total flow of FA to the small intestine (Boerman et al., 2015; Rico et al., 2017; de Souza et al., 2018). To better understand the mechanisms underlying FA absorption and limitations, more research is needed to examine the interactions between the amount and profile of FA reaching the duodenum.

We observed that compared with CON, CSPF increased milk yield by 1.53 kg/d, which is similar to previous observations by Rabiee et al. (2012) (1.55 kg/d), and Onetti and Grummer (2004) (1.29 kg/d). Some mechanisms may explain the positive effect of CSPF on milk yield. In general, FA inclusion increases energy efficiency in lactating cows by generating more ATP per mol than glucose and protein, by promoting nutrient partition toward milk production, and by sparing energy by decreasing de novo milk FA synthesis (Bauman and Davis, 1974; Palmquist, 1994, 2006). Also, FA have a high energy density that can be incorporated into the diet without having to considerably increase the heat increment (Chan et al., 1997; Wang et al., 2010). Other nutritional aspects could explain the positive effect of CSPF on milk yield. In our meta-regression, CSPF increased NDF digestibility and tended to increase FA digestibility for each 1-percentage-unit increase of CSPF in diet DM, increasing total FA absorption. In fact, CSPF had greater milk fat yield than CON, which was driven by the incorporation of mixed and preformed FA in milk fat. Other meta-analyses have reported that CSPF increased milk fat yield but did not report milk FA sources (Onetti and Grummer, 2004; Rabiee et al., 2012). In previous individual studies, CSPF also increased milk fat yield due to an increase in mixed and preformed milk FA (Schauff and Clark, 1992; de Souza and Lock, 2018a).

Microbial protein is the most important protein source for lactating dairy cows, because it provides a similar amino acid profile to milk protein, particularly its balance of lysine and methionine (Santos et al., 1998). Protein synthesis by bacterial cells depends on the availability of RDP and fermentable carbohydrates (Nocek and Russell, 1988; NRC, 2001). In our data set, diet CP content was on average 18% regardless of treatment, and CP digestibility was not affected by the inclusion of CSPF. Therefore, RDP was not limited in CSPF diets. Furthermore, CSPF increased NDF digestibility, which could have counterbalanced the reduction in DMI, resulting in microbial protein synthesis sufficient to provide MP similar to CON. This could support why CSPF did not affect milk protein yield. Other studies reported similar responses (de Souza et al., 2018; de Souza and Lock, 2018a). In CSPF supplementation studies, a reduction in milk protein content with no change in milk protein yield is a very common observation (de Souza et al., 2018; Oyebade et al., 2020). We observed this effect in our study, which could have been the consequence of milk component dilutions, because CSPF increased milk yield, but had no effect on milk protein yield.

The observed increase in yields of milk and milk fat resulted in CSPF also increasing 3.5% FCM yield and energy output to milk, and improved feed efficiency (ECM/DMI). However, increasing the yields of both milk and milk fat resulted in no effect of CSPF on milk fat content. Other studies have reported that CSPF induced a dilution effect on milk fat content (Onetti and Grummer, 2004; Oyebade et al., 2020). Interestingly, the inclusion of CSPF did not affect BW, BW change, BCS, or energy output to maintenance. Unfortunately, the current data set did not allow for a robust assessment of CSPF on energy output to body reserves, energy partitioning, and blood parameters. The effects of CSPF on energetic metabolism have been inconsistent across studies, which is associated with different diets, stages of lactation, physiological conditions, and heat stress. Batistel et al. (2017) and de Souza et al. (2017) reported that the inclusion of CSPF in a corn-based supplement for early-lactation grazing cows reduced BW change and energy partitioning to body reserves. On the other hand, replacing soybean hulls with CSPF-based FA blends increased BW change and energy partitioning to body reserves in mid-lactation cows fed corn silage-based TMR (de Souza et al., 2018, 2019). This positive response on energy partitioning to body reserves might be associated with the stimulatory effect of *cis*-9 C18:1 on pancreatic β-cells, resulting in increased insulin secretion (Fujiwara et al., 2005; de Souza et al., 2018). In addition, human-related studies have demonstrated that *cis*-9 C18:1 enhances insulin sensitivity of adipose tissue (Finucane et al., 2015), but this has not yet been extensively studied in ruminants. Further studies are needed to better understand the effects of specific FA on nutrient partitioning in lactating dairy cows.

Although CSPF increased milk fat yield compared with CON, it reduced the yield of de novo milk FA. Glasser et al. (2008) proposed an interdependence between milk FA, wherein preformed FA would stimulate an increase in de novo milk FA in cows fed low fat diets. However, when cows are fed high FA diets, they suggested an inverse relationship and described a substitution effect of de novo by preformed FA in milk fat. Therefore, our results indicate that CSPF promoted a substitution effect. Previous studies have also reported the same substitution effect in FA supplemented diets (He and Armentano, 2011; He et al., 2012). Elevated *trans*-10 C18:1 in milk fat is also associated with a reduction in de yield of novo FA (Dórea and Armentano, 2017). However, Lock et al. (2007) demonstrated that *trans*-10 C18:1 has no direct effect on the yield of de novo milk FA. Unfortunately, our data set did not include sufficient data to evaluate milk *trans*-FA. This was reported in only one study, and the most potent biohydrogenation intermediate that inhibits all milk FA synthesis (*trans*-10,*cis*-12 C18:2; Bauman et al., 2011) was not detected (de Souza and Lock, 2018a).

The mechanisms for FA substitution may be explained by the competition between de novo and exogenous long-chain FA to be incorporated onto the glycerol-3-phosphate backbone. Milk triglyceride synthesis is a highly coordinated process, and the location of FA along the glycerol backbone is not random, with individual FA being preferentially located at different positions by specific enzymes (Jensen, 2002; Lindmark Månsson, 2008). Distribution of C16:0 is uniform between the *sn*-1 (44.1%) and *sn*-2 (45.2%) positions of the glycerol backbone (Jensen, 2002). More than 50% of de novo milk FA from 8 to 14-carbons are also esterified at *sn*-2, whereas C18:1 is esterified at *sn*-1 (37.5%) and *sn*-3 (41.5%) positions. Over 98% of C4:0 and 93% of C6:0 are added at the *sn*-3 position (Jensen, 2002). We observed that CSPF decreased the yield of milk FA from 8 to 14-carbons, which is possibly a result of the increase in milk C16:0 yield at *sn*-2. Studies with enriched C16:0 supplements have also reported a reduction in the yields of milk FA from 8 to 14-carbons. In these studies, the yield of C4:0 increased, and the yield of C6:0 was not affected (Piantoni et al., 2013; de Souza and Lock, 2018b). In our study, however, CSPF did not affect milk C4:0 and decreased milk C6:0 yield. The esterification of *cis*-9 C18:1 at *sn*-3 may have decreased milk C6:0 yield but had no effect on milk C4:0 yield. Ruminants are unique in their incorporation of C4:0 into milk fat (Ashworth et al., 1966; Bauman and Griinari, 2003). Decreases in milk C4:0 yield are not common, because this FA has a low melting point (−5.3°C), helping to maintain milk fat fluidity at body temperature (Barbano and Sherbon, 1980; Scrimgeour and Harwood, 2007). Also, the pathway to generate C4:0 involves the use of a more efficient primer (butyryl-CoA) independent from malonyl-CoA, which spares ATP and NADPH (Palmquist et al., 1969; Lin and Kumar, 1972; Smith et al., 1974).

Linear effects to CSPF supplementation in the diets of lactating dairy cow have been evaluated in previous meta-regression studies, in which there was no limit on diet FA inclusion. Allen (2000) observed that DMI was reduced by approximately 2.5% for each 1-percentage-unit increase of CSPF in diet DM. Weld and Armentano (2017) reported that each 1-percentage-unit increase of CSPF in diet DM reduced DMI by 0.40 kg/d but had no effect on NDF digestibility. Onetti and Grummer (2004) observed that each 1-percentage-unit increase of CSPF in diet DM where the forage source was a mix of alfalfa and corn silage had no effect on milk yield, and increased the yield of milk fat by 10.0 g/d. Compared with these studies, we limited the inclusion rate of CSPF to ≤3% diet DM, and we observed greater values for milk yield (0.54 kg/d) and milk fat yield (25.6 g/d), a lower DMI reduction (0.37 kg/d), and an increase in NDF digestibility (1.15 percentage units) for each 1-percentage-unit increase of CSPF in diet DM. We also described the effect of increasing the inclusion of CSPF in diet DM on milk FA; increasing CSPF decreased the yield of de novo and increased the yields of mixed and preformed milk FA. A similar pattern was observed on a concentration basis. To our knowledge, no other meta-regression studies have evaluated CSPF inclusion on milk FA profile. In our data set, only one study reported the effects of increasing doses of CSPF (0, 0.25, 0.50, and 0.75 kg/d, Beaulieu and Palmquist, 1995). These authors observed that increasing CSPF linearly reduced the concentration of de novo milk FA from 8 to 14-carbons, linearly increased *cis*-9 C18:1 concentration, and had no effect on C16:0 concentration. Further studies are needed to more fully understand the effects of CSPF in the diets of lactating dairy cows.

CONCLUSIONS

Our results indicate no reason for the restrictive use of change-over designs in CSPF supplementation studies and meta-analyses. Feeding CSPF reduced DMI, increased NDF digestibility, tended to increase FA digestibility, increased the yields of milk, milk fat, and 3.5% FCM, decreased milk protein content, and improved feed efficiency. The increase in milk fat yield was driven by increases in the yields of mixed and preformed milk FA.

ACKNOWLEDGMENTS

We acknowledge the São Paulo Research Foundation (FAPESP, project # 2018/08016-7) for the scholarship granted to J. M. dos Santos Neto and the support provided by Global Agri-Trade (Rancho Dominguez, CA) and Perdue AgriBusiness (Salisbury, MD) to the Michigan State University Dairy Nutrition Graduate Fellowship Program, which also supported J. M. dos Santos Neto. We thank Normand St-Pierre (Perdue AgriBusiness) for discussions and advice regarding statistical analysis. The authors have not stated any conflicts of interest.

REFERENCES

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

Ashworth, U. S., G. D. Ramaiah, and M. C. Keyes. 1966. Species difference in the composition of milk with special reference to the northern fur seal. J. Dairy Sci. 49:1206–1211. https://doi.org/10.3168/jds.S0022-0302(66)88054-2.

Baker, W. L., C. Michael White, J. C. Cappelleri, J. Kluger, and C. I. Coleman. 2009. Understanding heterogeneity in meta-analysis: The role of meta-regression. Int. J. Clin. Pract. 63:1426–1434. https://doi.org/10.1111/j.1742-1241.2009.02168.x.

Barbano, D. M., and J. W. Sherbon. 1980. Polyunsaturated protected lipid: Effect on triglyceride molecular weight distribution. J. Dairy Sci. 63:731–740. https://doi.org/10.3168/jds.S0022-0302(80)83000-1.

Batistel, F., J. de Souza, and F. A. P. Santos. 2017. Corn grain-processing method interacts with calcium salts of palm fatty acids supplementation on milk production and energy balance of early lactation cows grazing tropical pasture. J. Dairy Sci. 100:5343–5357. https://doi.org/10.3168/jds.2016-12503.

Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. Pages 31–75 in Lactation: A Comprehensive Treatise. B. Larson, ed. Academic Press.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203–227. https://doi.org/10.1146/annurev.nutr.23.011702.073408.

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. https://doi.org/10.1146/annurev.nutr.012809.104648.

Beaulieu, A. D., and D. L. Palmquist. 1995. Differential effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. J. Dairy Sci. 78:1336–1344. https://doi.org/10.3168/jds.S0022-0302(95)76755-8.

Bettero, V. P., T. A. Del Valle, R. V. Barletta, C. E. de Araújo, E. Ferreira de Jesus, G. F. Almeida, C. S. Takiya, F. Zanferari, P. G. de Paiva, J. E. de Freitas Júnior, and F. P. Rennó. 2017. Use of protected fat sources to reduce fatty acid biohydrogenation and improve abomasal flow in dry dairy cows. Anim. Feed Sci. Technol. 224:30–38. https://doi.org/10.1016/j.anifeedsci.2016.12.007.

Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta regression. J. Dairy Sci. 98:8889–8903. https://doi.org/10.3168/jds.2015-9592.

Cant, J. P., A. H. Fredeen, T. MacIntyre, J. Gunn, and N. Crowe. 1997. Effect of fish oil and monensin on milk composition in dairy cows. Can. J. Anim. Sci. 77:125–131. https://doi.org/10.4141/A95-125.

Chan, S. C., J. T. Huber, K. H. Chen, J. M. Simas, and Z. Wu. 1997. Effects of ruminally inert fat and evaporative cooling on dairy cows in hot environmental temperatures. J. Dairy Sci. 80:1172–1178. https://doi.org/10.3168/jds.S0022-0302(97)76044-2.

Czerkawski, J. W., K. L. Blaxter, and F. W. Wainman. 1966. The effect of linseed oil and of linseed oil fatty acids incorporated in the diet on the metabolism of sheep. Br. J. Nutr. 20:485–494. https://doi.org/10.1079/BJN19660048.

Davison, K. L., and W. Woods. 1963. Effect of calcium and magnesium upon digestibility of a ration containing corn oil by lambs. J. Anim. Sci. 22:27–29. https://doi.org/10.2527/jas1963.22127x.

de Souza, J., F. Batistel, and F. A. P. Santos. 2017. Effect of sources of calcium salts of fatty acids on production, nutrient digestibility, energy balance, and carryover effects of early lactation grazing dairy cows. J. Dairy Sci. 100:1072–1085. https://doi.org/10.3168/jds.2016-11636.

de Souza, J., and A. L. Lock. 2018a. Short communication: Comparison of a palmitic acid-enriched triglyceride supplement and calcium salts of palm fatty acids supplement on production responses of dairy cows. J. Dairy Sci. 101:3110–3117. https://doi.org/10.3168/jds.2017-13560.

de Souza, J., and A. L. Lock. 2018b. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. J. Dairy Sci. 101:3044–3056. https://doi.org/10.3168/jds.2017-13946.

de Souza, J., and A. L. Lock. 2019. Milk production and nutrient digestibility responses to triglyceride or fatty acid supplements enriched in palmitic acid. J. Dairy Sci. 102:4155–4164. https://doi.org/10.3168/jds.2018-15690.

de Souza, J., C. L. Preseault, and A. L. Lock. 2018. Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. J. Dairy Sci. 101:172–185. https://doi.org/10.3168/jds.2017-13460.

de Souza, J., C. M. Prom, and A. L. Lock. 2021. Altering the ratio of dietary of palmitic and oleic acids affects nutrient digestibility, metabolism, and energy balance during the immediate postpartum in dairy cows. J. Dairy Sci. 104:2910–2923. https://doi.org/10.3168/jds.2020-19312.

de Souza, J., N. R. St-Pierre, and A. L. Lock. 2019. Altering the ratio of dietary C16:0 and cis-9 C18:1 interacts with production level in dairy cows: Effects on production responses and energy partitioning. J. Dairy Sci. 102:9842–9856. https://doi.org/10.3168/jds.2019-16374.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and R. S. Patton. 2005. Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88:983–993. https://doi.org/10.3168/jds.S0022-0302(05)72766-1.

Devendra, C., and D. Lewis. 1974. The interaction between dietary lipids and fiber in the sheep. 2. Digestibility studies. Anim. Sci. 19:67–76. https://doi.org/10.1017/S0003356100022583.

Dórea, J. R. R., and L. E. Armentano. 2017. Effects of common dietary fatty acids on milk yield and concentrations of fat and fatty acids in dairy cattle. Anim. Prod. Sci. 57:2224–2236. https://doi.org/10.1071/AN17335.

Dórea, J. R. R., E. A. French, and L. E. Armentano. 2017. Use of milk fatty acids to estimate plasma nonesterified fatty acid concentrations as an indicator of animal energy balance. J. Dairy Sci. 100:6164–6176. https://doi.org/10.3168/jds.2016-12466.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008a. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. J. Dairy Sci. 91:1334–1346. https://doi.org/10.3168/jds.2007-0607.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008b. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347–1360. https://doi.org/10.3168/jds.2007-0608.

Finucane, O. M., C. L. Lyons, A. M. Murphy, C. M. Reynolds, R. Klinger, N. P. Healy, A. A. Cooke, R. C. Coll, L. McAllan, K. N. Nilaweera, M. E. O'Reilly, A. C. Tierney, M. J. Morine, J. F. Alcala-Diaz, J. Lopez-Miranda, D. P. O'Connor, L. A. O'Neill, F. C. McGillicuddy, and H. M. Roche. 2015. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity. Diabetes 64:2116–2128. https://doi.org/10.2337/db14-1098.

Firkins, J. L., and M. L. Eastridge. 1994. Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production. J. Dairy Sci. 77:2357–2366. https://doi.org/10.3168/jds.S0022-0302(94)77178-2.

Fletcher, J. 2007. What is heterogeneity and is it important? BMJ 334:94–96. https://doi.org/10.1136/bmj.39057.406644.68.

Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. Br. J. Nutr. 23:249–263. https://doi.org/10.1079/BJN19690032.

Fujiwara, K., F. Maekawa, and T. Yada. 2005. Oleic acid interacts with GPR40 to induce Ca2+ signaling in rat islet beta-cells: Mediation by PLC and L-type Ca2+ channel and link to insulin release. Am. J. Physiol. Endocrinol. Metab. 289:E670–E677. https://doi.org/10.1152/ajpendo.00035.2005.

Glasser, F., A. Ferlay, M. Doreau, P. Schmidely, D. Sauvant, and Y. Chilliard. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91:2771–2785. https://doi.org/10.3168/jds.2007-0383.

Grainger, R. B., M. C. Bell, J. W. Stroud, and F. H. Baker. 1961. Effect of various cations and corn oil on crude cellulose digestibility by sheep. J. Anim. Sci. 20:319–322.

Harvatine, K. J., and M. S. Allen. 2006. Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. J. Dairy Sci. 89:1092–1103. https://doi.org/10.3168/jds.S0022-0302(06)72177-4.

Hawke, J. C., and J. A. Robertson. 1964. Studies in rumen metabolism. II - In vivo hydrolysis and hydrogenation of lipid. J. Sci. Food Agric. 15:283–289. https://doi.org/10.1002/jsfa.2740150503.

Hawke, J. C., and W. R. Silcock. 1969. Lipolysis and hydrogenation in the rumen. Biochem. J. 112:131–132. https://doi.org/10.1042/bj1120131.

He, M., and L. E. Armentano. 2011. Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. J. Dairy Sci. 94:2481–2491. https://doi.org/10.3168/jds.2010-3755.

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. https://doi.org/10.3168/jds.2011-4635.

Hu, W., J. P. Boerman, and J. M. Aldrich. 2017. Production responses of Holstein dairy cows when fed supplemental fat containing saturated free fatty acids: A meta-analysis. Asian-Austral. J. Anim. Sci. 30:1105–1116. https://doi.org/10.5713/ajas.16.0611.

Huhtanen, P., and M. Hetta. 2012. Comparison of feed intake and milk production responses in continuous and change-over design dairy cow experiments. Livest. Sci. 143:184–194. https://doi.org/10.1016/j.livsci.2011.09.012.

Ikwuegbu, O. A., and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Br. J. Nutr. 48:365–375. https://doi.org/10.1079/BJN19820120.

Jenkins, T. C., and W. C. Bridges Jr.. 2007. Protection of fatty acids against ruminal biohydrogenation in cattle. Eur. J. Lipid Sci. Technol. 109:778–789. https://doi.org/10.1002/ejlt.200700022.

Jenkins, T. C., and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J. Dairy Sci. 67:978–986. https://doi.org/10.3168/jds.S0022-0302(84)81396-X.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350. https://doi.org/10.3168/jds.S0022-0302(02)74079-4.

Lean, I. J., A. R. Rabiee, T. F. Duffield, and I. R. Dohoo. 2009. Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. J. Dairy Sci. 92:3545–3565. https://doi.org/10.3168/jds.2009-2140.

Lin, C. Y., and S. Kumar. 1972. Pathway for the synthesis of fatty acids in mammalian tissues. J. Biol. Chem. 247:604–606. https://doi.org/10.1016/S0021-9258(19)45745-1.

Lindmark Månsson, H. 2008. Fatty acids in bovine milk fat. Food Nutr. Res. 52:1821. https://doi.org/10.3402/fnr.v52i0.1821.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, M. Mouloungui, L. Candy, and D. E. Bauman. 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71–76. https://doi.org/10.1093/jn/137.1.71.

Loften, J. R., and S. G. Cornelius. 2004. Responses of supplementary dry, rumen-inert fat sources in lactating dairy cow diets. Appl. Anim. Sci. 20:461–469. https://doi.org/10.15232/S1080-7446(15)31350-4.

Loften, J. R., J. G. Linn, J. K. Drackley, T. C. Jenkins, C. G. Soderholm, and A. F. Kertz. 2014. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. J. Dairy Sci. 97:4661–4674. https://doi.org/10.3168/jds.2014-7919.

Mackie, R. I., B. A. White, and M. P. Bryant. 1991. Lipid metabolism in anaerobic ecosystems. Crit. Rev. Microbiol. 17:449–479. https://doi.org/10.3109/10408419109115208.

Moallem, U., G. Altmark, H. Lehrer, and A. Arieli. 2010. Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. J. Dairy Sci. 93:3192–3202. https://doi.org/10.3168/jds.2009-2979.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070–2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9.

NRC (National Research Council). 2001. Nutritional Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci.

Onetti, S. G., and R. R. Grummer. 2004. Response of lactating cows to three supplemental fat sources as affected by forage in the diet and stage of lactation: A meta-analysis of literature. Anim. Feed Sci. Technol. 115:65–82. https://doi.org/10.1016/j.anifeedsci.2004.02.009.

Oyebade, A., L. Lifshitz, H. Lehrer, S. Jacoby, Y. Portnick, and U. Moallem. 2020. Saturated fat supplemented in the form of triglycerides decreased digestibility and reduced performance of dairy cows as compared to calcium salt of fatty acids. Animal 14:973–982. https://doi.org/10.1017/S1751731119002465.

Page, M. J., J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow, L. Shamseer, J. M. Tetzlaff, E. A. Akl, S. E. Brennan, R. Chou, J. Glanville, J. M. Grimshaw, A. Hróbjartsson, M. M. Lalu, T. Li, E. W. Loder, E. Mayo-Wilson, S. McDonald, L. A. McGuinness, L. A. Stewart, J. Thomas, A. C. Tricco, V. A. Welch, P. Whiting, and D. Moher. 2021. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372:n71. https://doi.org/10.1136/bmj.n71.

Palmquist, D. L. 1991. Influence of source and amount of dietary fat on digestibility in lactating cows. J. Dairy Sci. 74:1354–1360. https://doi.org/10.3168/jds.S0022-0302(91)78290-8.

Palmquist, D. L. 1994. The role of dietary fats in efficiency of ruminants. J. Nutr. 124:1377S–1382S.

Palmquist, D. L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. Pages 43–92 in Advanced Dairy Chemistry. Springer. https://doi.org/10.1007/0-387-28813-9_2.

Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-) β-hydroxybutyrate. J. Dairy Sci. 52:633–638. https://doi.org/10.3168/jds.S0022-0302(69)86620-8.

Palmquist, D. L., and T. C. Jenkins. 2017. A 100-year review: Fat feeding of dairy cows. J. Dairy Sci. 100:10061–10077. https://doi.org/10.3168/jds.2017-12924.

Peters, J. L., A. J. Sutton, D. R. Jones, K. R. Abrams, and L. Rushton. 2008. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. J. Clin. Epidemiol. 61:991–996. https://doi.org/10.1016/j.jclinepi.2007.11.010.

Piantoni, P., A. L. Lock, and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143–7154. https://doi.org/10.3168/jds.2013-6680.

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. https://doi.org/10.3168/jds.2011-4895.

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. https://doi.org/10.3168/jds.S0022-0302(07)71636-3.

Rico, J. E., J. de Souza, M. S. Allen, and A. L. Lock. 2017. Nutrient digestibility and milk production responses to increasing levels of palmitic acid supplementation vary in cows receiving diets with or without whole cottonseed. J. Anim. Sci. 95:436–446. https://doi.org/10.2527/jas.2016.1089.

Rodney, R. M., P. Celi, W. Scott, K. Breinhild, and I. J. Lean. 2015. Effects of dietary fat on fertility of dairy cattle: A meta-analysis and meta-regression. J. Dairy Sci. 98:5601–5620. https://doi.org/10.3168/jds.2015-9528.

Rodrigues, J. P. P., R. M. de Paula, L. N. Rennó, G. P. Costa, V. C. E. Hamade, S. C. Valadares Filho, F. P. Rennó, and M. I. Marcondes. 2019. Effects of soybean oil supplementation on performance, digestion and metabolism of early lactation dairy cows fed sugarcane-based diets. Animal 13:1198–1207. https://doi.org/10.1017/S1751731118002781.

Rodriguez, L. A., C. C. Stallings, J. H. Herbein, and M. L. McGilliard. 1997. Effect of degradability of dietary protein and fat on ruminal, blood, and milk components of Jersey and Holstein cows. J. Dairy Sci. 80:353–363. https://doi.org/10.3168/jds.S0022-0302(97)75945-9.

Salfer, J. A., J. G. Linn, D. E. Otterby, W. P. Hansen, and C. G. Soderholm. 1994. Effects of calcium salts of long-chain fatty acids added to a diet containing choice white grease on lactation performance. J. Dairy Sci. 77:2367–2375. https://doi.org/10.3168/jds.S0022-0302(94)77179-4.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182–3213. https://doi.org/10.3168/jds.S0022-0302(98)75884-9.

Schauff, D. J., and J. H. Clark. 1989. Effects of prilled fatty acids and calcium salts of fatty acids on rumen fermentation, nutrient digestibilities, milk production, and milk composition. J. Dairy Sci. 72:917–927. https://doi.org/10.3168/jds.S0022-0302(89)79185-2.

Schauff, D. J., and J. H. Clark. 1992. Effects of feeding diets containing calcium salts of long-chain fatty acids to lactating dairy cows. J. Dairy Sci. 75:2990–3002. https://doi.org/10.3168/jds.S0022-0302(92)78063-1.

Schauff, D. J., J. H. Clark, and J. K. Drackley. 1992. Effects of feeding lactating dairy cows diets containing extruded soybeans and calcium salts of long-chain fatty acids. J. Dairy Sci. 75:3003–3019. https://doi.org/10.3168/jds.S0022-0302(92)78064-3.

Schneider, P., D. Sklan, W. Chalupa, and D. S. Kronfeld. 1988. Feeding calcium salts of fatty acids to lactating cows. J. Dairy Sci. 71:2143–2150. https://doi.org/10.3168/jds.S0022-0302(88)79787-8.

Scrimgeour, C. M., and J. L. Harwood. 2007. Fatty acid and lipid structure. Pages 15–50 in The Lipid Handbook. F. D. Gunstone, J. L. Harwood, and A. J. Dijkstra, ed. CRC Press. https://doi.org/10.1201/9781420009675.

Sears, A., O. Gonzalez, A. Alberto, A. Young, J. de Souza, A. Relling, and F. Batistel. 2020. Effect of feeding a palmitic acid–enriched supplement on production responses and nitrogen metabolism of mid-lactating Holstein and Jersey cows. J. Dairy Sci. 103:8898–8909. https://doi.org/10.3168/jds.2020-18232.

Simas, J. M., J. T. Huber, Z. Wu, K. H. Chen, S. C. Chan, C. B. Theurer, and R. S. Swingle. 1995. Influence of steam-flaked sorghum grain and supplemental fat on performance of dairy cows in early lactation. J. Dairy Sci. 78:1526–1533. https://doi.org/10.3168/jds.S0022-0302(95)76774-1.

Smith, G. H., S. McCarthy, and J. A. F. Rook. 1974. Synthesis of milk fat from β-hydroxybutyrate and acetate in lactating goats. J. Dairy Res. 41:175–191. https://doi.org/10.1017/S0022029900019609.

St-Pierre, N. R. 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741–755. https://doi.org/10.3168/jds.S0022-0302(01)74530-4.

St-Pierre, N. R. 2007. Meta-analyses of experimental data in the animal sciences. Rev. Bras Zootecn. 36:343–358. https://doi.org/10.1590/S1516-35982007001000031.

Sterne, J. A. C., A. J. Sutton, J. P. A. Ioannidis, N. Terrin, D. R. Jones, J. Lau, J. Carpenter, G. Rücker, R. M. Harbord, C. H. Schmid, J. Tetzlaff, J. J. Deeks, J. Peters, P. Macaskill, G. Schwarzer, S. Duval, D. G. Altman, D. Moher, and J. P. Td. Higgins. 2011. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 343:d4002. https://doi.org/10.1136/bmj.d4002.

Sterne, J. A. C., and M. Egger. 2001. Funnel plots for detecting bias in meta-analysis: Guidelines on choice of axis. J. Clin. Epidemiol. 54:1046–1055. https://doi.org/10.1016/S0895-4356(01)00377-8.

Sterne, J. A. C., and R. M. Harbord. 2004. Funnel plots in meta-analysis. Stata J. 4:127–141. https://doi.org/10.1177/1536867X0400400204.

Sukhija, P. S., and D. L. Palmquist. 1990. Dissociation of calcium soaps of long-chain fatty acids in rumen fluid. J. Dairy Sci. 73:1784–1787. https://doi.org/10.3168/jds.S0022-0302(90)78858-3.

Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Softw. 36:1–48. https://doi.org/10.18637/jss.v036.i03.

Vlaeminck, B., V. Fievez, A. J. R. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd-and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389–417. https://doi.org/10.1016/j.anifeedsci.2006.06.017.

Wang, J. P., D. P. Bu, J. Q. Wang, X. K. Huo, T. J. Guo, H. Y. Wei, L. Y. Zhou, R. R. Rastani, L. H. Baumgard, and F. D. Li. 2010. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. J. Dairy Sci. 93:4121–4127. https://doi.org/10.3168/jds.2009-2635.

Weld, K. A., and L. E. Armentano. 2017. The effects of adding fat to diets of lactating dairy cows on total-tract neutral detergent fiber digestibility: A meta-analysis. J. Dairy Sci. 100:1766–1779. https://doi.org/10.3168/jds.2016-11500.

Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025–3034. https://doi.org/10.3168/jds.S0022-0302(91)78488-9.

Zanton, G. I. 2019. Effect of experimental design on responses to 2 concentrations of metabolizable protein in multiparous dairy cows. J. Dairy Sci. 102:5094–5108. https://doi.org/10.3168/jds.2018-15730.

ORCIDS

J. M. dos Santos Neto https://orcid.org/0000-0002-5335-8933
J. de Souza https://orcid.org/0000-0002-7686-7079
A. L. Lock https://orcid.org/0000-0002-9282-399X

APPENDIX

List of Studies Used in the Data Set

Andrew, S. M., H. F. Tyrrell, C. K. Reynolds, and R. A. Erdman. 1991. Net energy for lactation of calcium salts of long-chain fatty acids for cows fed silage-based diets. J. Dairy Sci. 74:2588–2600. https://doi.org/10.3168/jds.S0022-0302(91)78437-3.

Beaulieu, A. D., and D. L. Palmquist. 1995. Differential effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. J. Dairy Sci. 78:1336–1344. https://doi.org/10.3168/jds.S0022-0302(95)76755-8 .

Canale, C. J., P. L. Burgess, L. D. Muller, and G. A. Varga. 1990. Calcium salts of fatty acids in diets that differ in neutral detergent fiber: Effect on lactation performance and nutrient digestibility. J. Dairy Sci. 73:1031–1038. https://doi.org/10.3168/jds.S0022-0302(90)78762-0.

Cervantes, A., T. R. Smith, and J. W. Young. 1996. Effects of nicotinamide on milk composition and production in dairy cows fed supplemental fat. J. Dairy Sci. 79:105–113. https://doi.org/10.3168/jds.S0022-0302(96)76340-3.

de Souza, J., and A. L. Lock. 2018a. Short communication: Comparison of a palmitic acid-enriched triglyceride supplement and calcium salts of palm fatty acids supplement on production responses of dairy cows. J. Dairy Sci. 101:3110–3117. https://doi.org/10.3168/jds.2017-13560.

DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and R. S. Patton. 2005. Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88:983–993. https://doi.org/10.3168/jds.S0022-0302(05)72766-1.

Erickson, P. S., M. R. Murphy, and J. H. Clark. 1992. Supplementation of dairy cow diets with calcium salts of long-chain fatty acids and nicotinic acid in early lactation. J. Dairy Sci. 75:1078–1089. https://doi.org/10.3168/jds.S0022-0302(92)77852-7.

Garcia-Bojalil, C. M., C. R. Staples, C. A. Risco, J. D. Savio, and W. W. Thatcher. 1998. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: Productive responses. J. Dairy Sci. 81:1374–1384. https://doi.org/10.3168/jds.S0022-0302(98)75701-7.

Garnsworthy, P. C., A. Lock, G. E. Mann, K. D. Sinclair, and R. Webb. 2008. Nutrition, metabolism, and fertility in dairy cows: 2. Dietary fatty acids and ovarian function. J. Dairy Sci. 91:3824–3833. https://doi.org/10.3168/jds.2008-1032.

Harrison, J. H., R. L. Kincaid, J. P. McNamara, S. Waltner, K. A. Loney, R. E. Riley, and J. D. Cronrath. 1995. Effect of whole cottonseeds and calcium salts of long-chain fatty acids on performance of lactating dairy cows. J. Dairy Sci. 78:181–193. https://doi.org/10.3168/jds.S0022-0302(95)76628-0.

Holter, J. B., H. H. Hayes, W. E. Urban Jr., and A. H. Duthie. 1992. Energy balance and lactation response in Holstein cows supplemented with cottonseed with or without calcium soap. J. Dairy Sci. 75:1480–1494. https://doi.org/10.3168/jds.S0022-0302(92)77905-3.

Kent, B. A., and M. J. Arambel. 1988. Effect of calcium salts of long-chain fatty acids on dairy cows in early lactation. J. Dairy Sci. 71:2412–2415. https://doi.org/10.3168/jds.S0022-0302(88)79826-4.

Moallem, U., G. Altmark, H. Lehrer, and A. Arieli. 2010. Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. J. Dairy Sci. 93:3192–3202. https://doi.org/10.3168/jds.2009-2979.

Moallem, U., Y. Folman, A. Bor, A. Arav, and D. Sklan. 1999. Effect of calcium soaps of fatty acids and administration of somatotropin on milk production, preovulatory follicular development, and plasma and follicular fluid lipid composition in high yielding dairy cows. J. Dairy Sci. 82:2358–2368. https://doi.org/10.3168/jds.S0022-0302(99)75486-X.

Moallem, U., Y. Folman, and D. Sklan. 2000. Effects of somatotropin and dietary calcium soaps of fatty acids in early lactation on milk production, dry matter intake, and energy balance of high-yielding dairy cows. J. Dairy Sci. 83:2085–2094. https://doi.org/10.3168/jds.S0022-0302(00)75090-9.

Moallem, U., M. Kaim, Y. Folman, and D. Sklan. 1997. Effect of calcium soaps of fatty acids and administration of somatotropin in early lactation on productive and reproductive performance of high producing dairy cows. J. Dairy Sci. 80:2127–2136. https://doi.org/10.3168/jds.S0022-0302(97)76158-7.

Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. J. Dairy Sci. 97:3739–3751. https://doi.org/10.3168/jds.2013-7341.

Rodriguez, L. A., C. C. Stallings, J. H. Herbein, and M. L. McGilliard. 1997. Effect of degradability of dietary protein and fat on ruminal, blood, and milk components of Jersey and Holstein cows. J. Dairy Sci. 80:353–363. https://doi.org/10.3168/jds.S0022-0302(97)75945-9.

Salfer, J. A., J. G. Linn, D. E. Otterby, W. P. Hansen, and C. G. Soderholm. 1994. Effects of calcium salts of long-chain fatty acids added to a diet containing choice white grease on lactation performance. J. Dairy Sci. 77:2367–2375. https://doi.org/10.3168/jds.S0022-0302(94)77179-4.

Schauff, D. J., and J. H. Clark. 1989. Effects of prilled fatty acids and calcium salts of fatty acids on rumen fermentation, nutrient digestibilities, milk production, and milk composition. J. Dairy Sci. 72:917–927. https://doi.org/10.3168/jds.S0022-0302(89)79185-2.

Schauff, D. J., and J. H. Clark. 1992. Effects of feeding diets containing calcium salts of long-chain fatty acids to lactating dairy cows. J. Dairy Sci. 75:2990–3002. https://doi.org/10.3168/jds.S0022-0302(92)78063-1.

Schauff, D. J., J. H. Clark, and J. K. Drackley. 1992. Effects of feeding lactating dairy cows diets containing extruded soybeans and calcium salts of long-chain fatty acids. J. Dairy Sci. 75:3003–3019. https://doi.org/10.3168/jds.S0022-0302(92)78064-3.

Schneider, P., D. Sklan, W. Chalupa, and D. S. Kronfeld. 1988. Feeding calcium salts of fatty acids to lactating cows. J. Dairy Sci. 71:2143–2150. https://doi.org/10.3168/jds.S0022-0302(88)79787-8.

Simas, J. M., J. T. Huber, Z. Wu, K. H. Chen, S. C. Chan, C. B. Theurer, and R. S. Swingle. 1995. Influence of steam-flaked sorghum grain and supplemental fat on performance of dairy cows in early lactation. J. Dairy Sci. 78:1526–1533. https://doi.org/10.3168/jds.S0022-0302(95)76774-1.

Sklan, D., R. Ashkenazi, A. Braun, A. Devorin, and K. Tabori. 1992. Fatty acids, calcium soaps of fatty acids, and cottonseeds fed to high yielding cows. J. Dairy Sci. 75:2463–2472. https://doi.org/10.3168/jds.S0022-0302(92)78008-4.

Sklan, D., M. Kaim, U. Moallem, and Y. Folman. 1994. Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows. J. Dairy Sci. 77:1652–1660. https://doi.org/10.3168/jds.S0022-0302(94)77107-1.

Sklan, D., U. Moallem, and Y. Folman. 1991. Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J. Dairy Sci. 74:510–517. https://doi.org/10.3168/jds.S0022-0302(91)78198-8.

Spicer, L. J., R. K. Vernon, W. B. Tucker, R. P. Wettemann, J. F. Hogue, and G. D. Adams. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. J. Dairy Sci. 76:2664–2673. https://doi.org/10.3168/jds.S0022-0302(93)77602-X.

Stoffel, C. M., P. M. Crump, and L. E. Armentano. 2015. Effect of dietary fatty acid supplements, varying in fatty acid composition, on milk fat secretion in dairy cattle fed diets supplemented to less than 3% total fatty acids. J. Dairy Sci. 98:431–442. https://doi.org/10.3168/jds.2014-8328.

Umphrey, J. E., B. R. Moss, C. J. Wilcox, and H. H. Van Horn. 2001. Interrelationships in lactating Holsteins of rectal and skin temperatures, milk yield and composition, dry matter intake, body weight, and feed efficiency in summer in Alabama. J. Dairy Sci. 84:2680–2685. https://doi.org/10.3168/jds.S0022-0302(01)74722-4.

Weiss, WP, and D. J. Wyatt. 2004. Digestible energy values of diets with different fat supplements when fed to lactating dairy cows. J. Dairy Sci. 87:1446–1254. https://doi.org/10.3168/jds.S0022-0302(04)73295-6.

Wu, Z., J. T. Huber, F. T. Sleiman, J. M. Simas, K. H. Chen, S. C. Chan, and C. Fontes. 1993. Effect of three supplemental fat sources on lactation and digestion in dairy cows. J. Dairy Sci. 76:3562–3570. https://doi.org/10.3168/jds.S0022-0302(93)77695-X.

Ylioja, C. M., C. Abney-Schulte, R. Stock, and B. J. Bradford. 2018. Effects of fat supplementation to diets high in nonforage fiber on production responses of midlactation dairy cows. J. Dairy Sci. 101:6066–6073. https://doi.org/10.3168/jds.2017-13991.