University of São Paulo
"Luiz de Queiroz" College of Agriculture

Effects of calcium salts of palm fatty acids on nutrient digestibility, energy partitioning and production responses of lactating dairy cows

José Maurício dos Santos Neto

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

Piracicaba
2020

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Animal Science

Effects of calcium salts of palm fatty acids on nutrient digestibility, energy partitioning and production responses of lactating dairy cows

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
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RESUMO

Efeitos dos sais de cálcio do óleo de palma sobre a digestibilidade dos nutrientes, partição energética, e respostas produtivas de vacas leiteiras em lactação

Objetivou-se a partir desta tese avaliar os efeitos de sais de cálcio dos ácidos graxos do óleo de palma (CSPF) sobre a digestibilidade dos nutrientes, o particionamento de energia e as respostas produtivas de vacas leiteiras em lactação. Uma meta-análise foi realizada para avaliar os efeitos dos CSPF em comparação com dietas controle sem inclusão de gordura sobre a digestibilidade dos nutrientes e as respostas produtivas de vacas leiteiras em lactação recebendo dieta total (Capítulo 2). Além disso, também utilizamos uma meta-análise para avaliar se os delineamentos experimentais contínuos (inteiramente casualizados, ou casualizados em blocos) ou não contínuos (quadrado latino ou crossover) afetam as repostas obtidas pela suplementação com CSPF. Mais dois experimentos foram realizados para avaliar os efeitos dos CSPF sobre a digestibilidade dos nutrientes, o particionamento de energia e as respostas produtivas de vacas leiteiras em lactação mantidas em pastagens tropicais. Em um dos experimentos, os CSPF foram avaliados em vacas leiteiras no meio da lactação (Capítulo 3), e no outro experimento foram avaliados os efeitos de níveis de inclusão de CSPF em vacas leiteiras no início da lactação, com consequente avaliação do efeito residual (Capítulo 4). A meta-análise indicou que os CSPF reduzem o CMS, aumentam a digestibilidade de FDN, e melhoraram as respostas produtivas de vacas leiteiras recebendo uma dieta total. Adicionalmente, não foram observadas interações entre os delineamentos experimentais e a suplementação com CSPF (Capítulo 2). O uso de CSPF para vacas leiteiras no meio da lactação mantidas em pastagens tropicais reduziu o consumo de matéria orgânica, sem afetar a ingestão de energia, aumentou a digestibilidade da matéria orgânica, de FDN, e de ácidos graxos totais, além de ter alterado a partição energética, promovendo aumentos na produção de leite, deposição de gordura no leite, produção de leite corrigida para 3,5% de gordura, e também a produção de leite corrigida para energia (Capítulo 3). Os níveis de CSPF para vacas leiteiras no início da lactação mantidas em pastagens tropicais reduziram o CMS sem afetar consumo de energia, aumentaram linearmente a digestibilidade de FDN, e promoveram aumento quadrático sobre a digestibilidade de ácidos graxos totais, além de terem aumentado linearmente a produção de leite, a deposição de gordura no leite, e a produção de leite corrigida para 3,5% de gordura. O uso de CSPF para vacas leiteiras no início da lactação mantidas em pastagens tropicais acarretou em efeito residual positivo após o fim do período de suplementação (Capítulo 4). No geral, os resultados obtidos nesta tese indicam que as respostas obtidas por delineamentos contínuos e não contínuos à suplementação com CSPF são as mesmas. O uso de CSPF promove redução no CMS sem afetar a ingestão de energia, aumenta a digestibilidade de FDN e de ácidos graxos totais, promove alterações na partição de energia, aumentando a produção de leite, a deposição de gordura no leite, e a produção de leite corrigida para 3,5% de gordura. Quando fornecidos no início da lactação, os CSPF têm efeito residual positivo sobre a produção de leite após o término do período de suplementação.

Palavras-chaves: Gordura inerte; Meta-análise; Pasto tropical; Vacas em pastejo

ABSTRACT

Effects of calcium salts of palm fatty acids on nutrient digestibility, energy partitioning and production responses of lactating dairy cows

The objective of this thesis was to evaluate the effects of calcium salts of palm fatty acids (CSPF) on nutrient digestibility, energy partitioning and production responses of lactating dairy cows. A meta-analysis was performed to evaluate the effects of CSPF compared to non-fat supplemented control diets on nutrient digestibility and production responses of lactating dairy cows receiving TMR. In addition, we also used a meta-analysis to evaluate whether experimental designs classified as either continuous (completely randomized and randomized as block) or change-over (crossover and Latin square) impact the responses of supplemental CSPF (Chapter 2). More two studies were performed to evaluate the effects of CSPF on nutrient digestibility, energy partitioning, and production responses of lactating dairy cows grazing on tropical pastures. One experiment evaluated CSPF in mid-lactation dairy cows (Chapter 3), and the other evaluated the dose response effects of CSPF in early-lactation dairy cows, with a potentially positive carryover effect (Chapter 4). The meta-analysis indicated that CSPF increased NDF digestibility and improved the production responses of lactating dairy cows receiving TMR. In addition, we did not observe interactions between experimental designs and CSPF supplementation (Chapter 2). Feeding CSPF to mid-lactation dairy cows grazing on tropical pasture decreased OM intake, but did not affect energy intake, increased OM, NDF and fatty acid (FA) digestibility, and altered energy partitioning, promoting increases in the yields of milk, milk fat, 3.5% FCM, and ECM (Chapter 3). Increasing CSPF to early-lactation dairy cows grazing on tropical pastures linearly decreased DMI, but did not affect energy intake, linearly increased NDF and quadratically increased FA digestibility, and linearly increased energy output for milk, promoting linear increases in the yields of milk, milk fat, 3.5% FCM, and ECM. Feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production (Chapter 4). Our results indicate no reason for the restrictive use of change-over designs in CSPF supplementation studies and meta-analyses. Feeding CSPF to lactating dairy cows reduced DMI, but did not affect energy intake, increased NDF and FA digestibility, and altered energy partitioning, promoting increases in the yields of milk, milk fat, and 3.5% FCM. Also, feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production.

Keywords: Inert fat; Meta-analysis; Tropical pastures; Grazing cows

1. INTRODUCTION

The use of fat supplements is a common nutritional practice to increase the energy density of diets for lactating dairy cows. The interest in rumen inert fats, thought to have minimal impacts on rumen fermentation, has greatly increased over the years because of the positive responses that these fat supplements have on the production responses of lactating dairy cows (Palmquist and Jenkins, 2017).

Calcium salts of palm fatty acids (CSPF) are one of the most common rumen inert fats used in dairy cow nutrition, and are mainly composed of palmitic (C16:0; ~45%) and oleic (*cis*-9 C18:1; ~35%) acids (Loften and Cornelius, 2004; de Souza et al., 2019). They are developed through ionic bonds formed between hydrolyzed FA from palm fatty acids (FA) and calcium ions. Thus, CSPF are insoluble at rumen pH, but they are dissociated at the low pH of abomasum, making the FA available for absorption in the small intestine (Jenkins and Palmquist, 1982; Schneider et al., 1988).

A recent meta-analysis by Rabiee et al. (2012) demonstrated that CSPF decreased DMI and increased milk yield, but in this study there was no limit on fat inclusion, so some of the reported CSPF doses were higher than those commonly used on farms. Furthermore, Crossover and Latin square studies were excluded from this meta-analysis, because of the concerns related with potential carryover effects from one period to another. Due to this, several meta-analyses have used only studies with continuous designs (Duffield et al., 2008; Duffield et al., 2008a; Rodney et al., 2015). However, if this criterion of exclusion can be applied to meta-analyses, this should also invalidate the use of crossover and Latin square designs in all animal nutrition trials. Thus, a wealth of research information would be wasted (Hu et al., 2017).

Overall, research on grazing cows fed supplemental fat is scarce, but reviews about this subject have indicated that feeding supplemental fat decreased or had no effect on the

DMI of grazing cows, and at the same time, increased milk and FCM, suggesting an improvement in energy efficiency. Nevertheless, most of these reported studies are based on the inclusion of oilseeds, pure oil, very high fat levels, or fat sources with low digestibility (Bargo et al., 2003; Schroeder et al., 2004).

Recent findings with early-lactation dairy cows grazing on tropical pastures observed that CSPF was an effective strategy to alter energy partitioning, promoting increases in milk production, and milk yield components (Batistel et al., 2017; de Souza et al., 2017). In addition, these authors also observed a positive carryover effect at the end of the supplementation period from 13 up until 16 wk postpartum. Despite these positive effects, to our knowledge, no studies were designed to evaluate the effects of CSPF supplementation in mid-lactation dairy cows grazing on tropical pastures, or the effects of a dose response of CSPF in early-lactation dairy cows grazing on tropical pastures and its potential carryover effect.

Therefore, the objective of this thesis was to evaluate the overall responses of lactating dairy cows to CSPF supplementation by a meta analytical approach; to evaluate whether experimental designs impact production responses of supplemental CSPF by a meta analytical approach; to evaluate the effects of CSPF supplementation on nutrient digestibility, energy partitioning, and production responses of mid-lactation dairy cows grazing on tropical pastures; and to evaluate the dose response effects of CSPF on nutrient digestibility, energy partitioning and production responses of early-lactation dairy cows grazing on tropical pastures with a potentially positive carryover effect.

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2. EFFECTS OF CALCIUM SALTS OF PALM FATTY ACIDS ON NUTRIENT DIGESTIBILITY, AND PRODUCTION RESPONSES OF LACTATING DAIRY COWS: A META-ANALYSIS AND META-REGRESSION

INTERPRETATIVE SUMMARY

Effects of calcium salts of palm fatty acids on nutrient digestibility, and production responses of lactating dairy cows: A meta-analysis and meta-regression. Our primary objective was to perform a meta-analysis and meta-regression to evaluate the effects of calcium salts of palm fatty acids (**CSPF**) compared to non-fat supplemented control diets (**CON**) on nutrient digestibility and production responses of lactating dairy cows. Secondarily, our objective was to use a meta-analysis to evaluate whether experimental designs impact production responses of supplemental CSPF. Our results indicate no reason for the restrictive use of change-over designs in CSPF supplementation studies and meta-analysis. Feeding CSPF reduced DMI, increased NDF digestibility, increased the yields of milk, milk fat and 3.5% FCM, and decreased protein content. The increase in milk fat yield was driven by the increase in the yields of mixed and preformed milk fatty acids (**FA**).

ABSTRACT

Our primary objective was to perform a meta-analysis and meta-regression to evaluate the effects of calcium salts of palm fatty acids (**CSPF**) compared to non-fat supplemented control diets (**CON**) on nutrient digestibility and production responses of lactating dairy cows. Secondarily, our objective was to perform a meta-analysis to evaluate whether experimental designs impact production responses of supplemental CSPF. The database was formed from 33 peer-reviewed publications with CSPF supplemented at ≤ 3% diet DM. We used a 2 × 2 interaction between experimental designs (continuous vs. change-over) and treatments (CON vs. CSPF) to evaluate whether experimental designs impact supplemental CSPF. We evaluated the effects of CSPF compared with CON on nutrient digestibility and production responses of lactating dairy cows by a meta-analysis and meta-regression regardless of experimental design. There was no interaction between treatments and experimental design for any variable. Compared with CON, CSPF reduced DMI, increased NDF digestibility, increased the yields of milk, milk fat, and 3.5% FCM, and reduced the content of milk protein. There were no treatment differences for the yield of milk protein, for the content of milk fat, or for BW, BW change and BCS. Compared with CON, CSPF reduced the yield of de novo milk fatty acids (**FA**) and increased the yields of mixed and preformed milk FA. Our results indicate no reason for the restrictive use of change-over designs in CSPF supplementation studies and meta-analysis. Feeding CSPF increased NDF digestibility and improved the production responses of lactating dairy cows. Also, CSPF increased milk fat yield by increasing the yields of mixed and preformed milk FA.

Keywords: Calcium salts; Experimental design; Meta-analysis

2.1. Introduction

The importance of fat supplementation has been increasing over the years in the dairy cow industry. In a recent review, Palmquist and Jenkins (2017) described the historical progress of fat feeding in dairy cows, starting from the use of fat naturally present in feeds, to the commercially available fat supplements thought to have minimal impacts on rumen fermentation.

Research with sheep found that the dietary inclusion of cations can alleviate the negative effects of unsaturated fatty acids (FA) on DM and cellulose digestibility (Grainger et al., 1961; Davison and Woods, 1963). In addition, studies on ruminal lipolysis and hydrogenation indicated that only free carboxyl groups would have the potential to be harmful for rumen microorganisms (Hawke and Robertson, 1964; Hawke and Silcock, 1969). As a result, calcium salts of FA were developed through ionic bonds between hydrolyzed FA and calcium ions. The overall idea is that calcium salts of FA are insoluble at the rumen pH, but they are dissociated at the low pH of abomasum, making the FA available for absorption in the small intestine (Jenkins and Palmquist, 1982; 1984; Schneider et al., 1988; Loften and Cornelius, 2004).

Sukhija and Palmquist (1990) studied the dissociation pattern of calcium salts of FA from various fat sources in rumen fluid. They reported that calcium salts of unsaturated FA had high rumen dissociation, whereas calcium salts of palm FA (CSPF) were satisfactorily stable to a pH of 5.5. Calcium salts of palm FA are one of the most common rumen inert fats used in dairy cow nutrition, and are mainly composed of palmitic (C16:0; ~45%) and oleic (cis-9 C18:1; ~35%) acids (Loften and Cornelius, 2004; de Souza et al., 2019). A recent meta-analysis by Rabiee et al. (2012), which evaluated fat additions on lactating dairy cows, demonstrated that compared with a non-fat supplemented control diet (CON), CSPF decreased DMI and increased milk yield. However, in this study there was no limit on fat

inclusion, so some of the reported CSPF doses were higher than those commonly used on farms. As well, the authors didn't provide any information on digestibility and milk FA composition. Crossover and Latin square studies were excluded from this meta-analysis, because of the concerns related with potential carryover effects from one period to another. Due to this, several meta-analyses have used only studies with continuous designs (Duffield et al., 2008; Duffield et al., 2008a; Rodney et al., 2015).

Lean et al. (2009) supported that Latin square designs should be avoided in meta-analyses, by citing a feeding fish oil and monensin study to lactating dairy cows (Cant et al., 1997). According to them, 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 should also invalidate the use of crossover and Latin square designs in all animal nutrition trials. Nonetheless, 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 a fair hypothesis-testing. Thereby, the most appropriate assessment consists of verifying whether the responses to fat supplementation obtained in change-over designs follow the same pattern as the responses obtained in continuous designs.

Therefore, our primary objective was to perform a meta-analysis and meta-regression to evaluate the effects of CSPF compared with CON diets on nutrient digestibility and production responses of lactating dairy cows. Secondarily, our objective was to perform a meta-analysis to evaluate whether experimental designs impact production responses of supplemental CSPF.

2.2. Materials and methods

2.2.1. Study selection and data set

To perform this study, we searched for peer-reviewed papers that contained at least a comparison between a non-fat supplemented control diet (CON) with a calcium salts of palm fatty acids (CSPF) diet. The CSPF had to be included at ≤ 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 the electronic database (PubMed, Google Scholar, Science Direct, Scirus, CAB, and Elsevier) and in the search engines of Journal of Dairy Science, Journal of Animal Science, Animal, Animal Feed Science and Technology, Animal Production Science, Brazilian Journal of Animal Science, Journal of Animal Physiology and Animal Nutrition, Journal of the Science of Food and Agriculture, Livestock Science, and The Professional Animal Scientist. We also reviewed the citations from the previous systematic reviews related to CSPF supplementation (Firkins and Eastridge, 1994; Allen, 2000; Loften and Cornelius, 2004; Onetti and Grummer, 2004; Rabiee et al., 2012; Boerman et al., 2015; Dórea et al., 2017; Dórea and Armentano, 2017; Weld and Armentano, 2017). Our final data set consisted of 33 studies published between 1988 and 2019. The list of the studies used is in Table 1.

2.2.2. Data and calculations

Two meta-analyses and one meta-regression were performed. In the first meta-analysis (Meta.1), we evaluated whether experimental designs impact nutrient digestibility and production responses of supplemental CSPF. Thus, experimental designs were classified as either continuous (completely randomized and randomized as block) or change-over (crossover and Latin square), and treatments were CON and CSPF. We tested a 2 × 2 interaction between experimental design and treatment. A description of the experimental designs used by the selected papers is in Table 1. In the second meta-analysis (Meta.2), we evaluated the effects of CSPF compared with CON (treatments) on nutrient digestibility and

production responses of lactating dairy cows, regardless of experimental design. We also performed a meta-regression to evaluate a dose response of CSPF on nutrient digestibility and production responses of lactating dairy cows (Meta.3). In Meta.3 we calculated the difference between CSPF means minus CON means, which resulted in one observation per CSPF-CON pair (Δtreatments). Then, we used Δtreatments as the dependent variable (Y) and CSPF inclusion in diet (%DM) was used as the independent variable (X).

Some values were incomplete or not uniformly reported among the studies, which required the following calculations. Milk CP was converted to milk true protein (Schauff and Clark, 1989; Schauff and Clark, 1992; Schauff et al., 1992; DeFrain et al., 2005), considering that no-protein nitrogen represents approximately 6% of milk CP (NRC, 2001; Hu et al., 2017). For studies that reported EE instead of FA, total FA content of the diet (Schneider et al et al., 1988; Simas et al., 1995; Salfer et al., 1994; Rodriguez et al., 1997; Moallem et al., 2010) and total FA digestibility (Simas et al., 1995) were 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:

FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)]

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

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). The summation of milk FA concentrations and yields by source (de novo [Σ < C16], mixed [Σ C16 + C16:1], preformed [Σ > C16]), and saturation (Σ SFA, Σ MUFA, and Σ PUFA) were also calculated. Milk odd-branched-chain fatty acids were not used in the summation by source.

Energy output (Mcal/d) for milk and maintenance were calculated according to NRC (2001) as: 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%; and Maintenance energy output (Mcal/d) = 0.08 × kg $BW^{0.75}$.

Descriptive statistics for FA composition of CSPF supplements, nutrient composition of 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 2, 3, 4 and 5 respectively. Descriptive statistics for individual FA used to evaluate the effects of CSPF (Meta.2 and Meta.3) are shown in Supplementary Table 1.

The results from Meta.1 are reported in the tables as the mean difference between change-over and continuous design, and the results from Meta.2 are reported in the tables as the mean difference between CSPF and CON.

2.2.3. Weighing 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 divided by the mean of all weights. As a result, we were able to maintain the expressions of dispersion in the original scale of the measurements (St-Pierre, 2001; St-Pierre, 2007). For dependent variables that were calculated, we used the number of experimental units rather than standard error to define the weighing factors.

2.2.4. Statistical Analysis

In Meta.1, we used the following model: $Yijk = \mu + Ti + Ej + Bk + Ti \times Ej + Bk \times Ti + eijk$, where Yijk = dependent variable, μ = overall mean, Ti = fixed effect of treatments, Ej = fixed effect of experimental designs, Bk = random effect of study, Ti × Ej = fixed effect of interaction between treatments and experimental design, Bk × Ti = random effect of interaction between study and treatments, and eijk = residual error.

In Meta.2, we used the following model: Yik = μ + Ti + Bk + Bk × Ti + eik, where Yik = dependent variable, μ = overall mean, Ti = fixed effect of treatments, Bk = random effect of study, Bk × Ti = random effect of interaction between study and treatments, and eik = residual error.

In Meta.3, the intercepts were forced through zero, because at 0% CSPF in diet (%DM) there is no correspondent value for Δtreatments. This assumption was also used by Weld and Armentano (2017). Therefore, we utilized the following model for linear terms: Yik = DiCi + Bk + eik, where Yik = dependent variable, DiCi = slope of the Δtreatments due to CSPF in the diet (%DM), Bk = random effect of study, and eik = residual error.

All data was analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute, Cary, NC). WEIGHT statement was used to provide a weight for each observation in the input data set. In both Meta.1 and Meta.2, Bk × Ti was kept in the model only when its estimate in the covariance parameter was higher than zero. The equations from Meta.3 were adjusted to linear or quadratic models with the intercept forced to zero by the NOINT option. To adjust the observations for the lost dimension, avoiding regression bias in graphic representation, figures of study-adjusted values for Meta.3 were developed based on SAS code described by St-Pierre (2001). Differences between means in Meta.1 and 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 $P \leq 0.10$.

2.3. Results

2.3.1. Meta.1. Impact of experimental designs on nutrient digestibility and production responses of lactating dairy cows supplemented with CSPF

There was no interaction between treatments and experimental designs for any variable ($P > 0.05$, Table 6-7). Compared with continuous, change-over design increased the content of milk protein ($P = 0.02$, Table 6), and tended to increase the yield of mixed milk FA (P = 0.06, Table 7). Experimental design did not affect any other variables ($P > 0.05$, Table 6-7).

2.3.2. Meta.2. Nutrient digestibility and production responses of lactating dairy cows when CSPF are included in the diet

DMI and Nutrient Digestibility

Compared with CON, CSPF reduced DMI ($P = 0.01$), had no effect on DM ($P = 0.80$), CP ($P = 0.32$) or FA digestibility ($P = 0.12$), and increased NDF digestibility ($P = 0.01$, Table 8).

Production Responses

Compared with CON, CSPF increased the yields of milk ($P < 0.01$), 3.5% FCM ($P = 0.04$), milk fat ($P = 0.04$), and energy output for milk ($P = 0.01$). It tended to increase ECM ($P = 0.07$), and reduced the content of milk protein ($P = 0.02$, Table 9). There were no treatment differences for the yields of milk protein (P = 0.94) or for the contents of milk fat ($P = 0.43$), as well as for BW (P = 0.36), BW change (P = 0.25) or BCS (P = 0.15), and energy output for maintenance ($P = 0.45$, Table 9).

Milk Fatty Acid Concentration and Yield

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 yield of de novo milk FA ($P <$

0.01, Table 10), which was driven by the reduction in the yield of C6:0 ($P = 0.02$), C8:0 ($P < 0.01$), C10:0 ($P < 0.01$), C12:0 ($P < 0.01$), C14:0 ($P < 0.01$), and C14:1 milk FA ($P < 0.01$, Supplementary Table 2). There was no treatment effect on the yield of C4:0 milk FA ($P = 0.12$, Supplementary Table 2). Compared with CON, CSPF increased the yield of mixed milk FA ($P = 0.01$), wherein we observed an increase in C16:0 ($P = 0.02$) and no effect on C16:1 milk FA ($P = 0.44$, Supplementary Table 2). CSPF also increased the yields of preformed ($P < 0.01$, Table 10) and milk MUFA ($P < 0.01$, Supplementary Table 2) when compared with CON, primarily due to the increase in the yield of total C18:1 milk FA ($P < 0.01$, Supplementary Table 2).

We observed a similar pattern of results for milk FA on a content basis (g/100g) compared with a yield basis (g/d) (Table 10, Supplementary Table 2). The unique difference is that CSPF increased the content of C4:0 milk FA ($P = 0.03$).

2.3.3. Meta.3. A dose response of CSPF on nutrient digestibility and production responses of lactating dairy cows

The inclusion of CSPF ranged from 0.78 to 3.00%, with an average of 2.20% in diet DM (Table 2). We analyzed linear and quadratic relationships between CSPF inclusion and Δtreatments. As previously explained, the intercepts were forced through zero, which represents no fat addition or CON (Weld and Armentano, 2017). Furthermore, intercepts were initially tested and not found to be significant or to have tendencies ($P > 0.10$, data not shown).

We observed a negative linear effect for DMI ($P < 0.01$), with a decrease of 0.22 kg/d for each percentage unit of added CSPF in the diet (%DM), as well as a positive linear effect for NDF digestibility ($P = 0.05$, Figure 1), with an increase of 0.58% percent units for each percentage unit of added CSPF in the diet (%DM). Regarding production responses, we observed positive linear effects for the yields of milk, milk fat, and 3.5% FCM ($P < 0.01$,

Figure 2), with increases of 0.42, 0.02, and 0.44 kg/d for each percentage unit of added CSPF in the diets (%DM) respectively. Also, there was a tendency for a linear increase in ECM (P = 0.06, Supplemental Figure 1), and a linear decrease of 0.02 g/100g in milk protein content (P < 0.01, Supplemental Figure 2).

De novo milk FA linearly decreased (P < 0.01) by 18.4 g/d for each percentage unit of added CSPF in the diet (%DM), at the same time as mixed milk FA and preformed milk FA linearly increased (P < 0.01, Figure 3) by 6.80, and 20.0 g/d for each percentage unit of added CSPF in the diets (%DM) respectively.

2.4. Discussion

Previous systematic reviews have shed light on the positive effects of added CSPF in the diet of lactating dairy cows (Allen, 2000; Onetti and Grummer, 2004; Rabbie et al., 2012; Weld and Armentano, 2017). However, in these studies there was no limit on fat inclusion, so that some of the reported doses were higher than those commonly used on farms. Additionally, serious consideration has been given to the appropriateness of including change-over designs in meta-analyses (Lean et al., 2009). 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. We also performed another meta-analysis to evaluate whether experimental designs impact the responses of supplemental CSPF.

Concerns with change-over designs include mostly 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 the meta-analysis performed to evaluate the effects of fat additions to dairy cattle diets. In our results, however, no interactions between experimental

designs and treatments were observed for any variable, clearly indicating a no dependence relationship among the components of these fixed factors. Therefore, the results obtained for CSPF responded in the same direction in both continuous and change-over designs. Similarly, in a recent meta-analysis with lactating dairy cows, Hu et al. (2017) observed that the responses to supplemental saturated fat were the same regardless of experimental design. These authors also pointed out that change-over designs are built on the assumption of no carryover effect 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. Likewise, Zanton (2019) reported that most responses to differences in MP of dairy cow diets are consistent between trials randomized as block or as Latin square design, and the obtained results go against the belief that change-over wouldn't promote detectable effects due to short experimental periods. The same is supported by the findings from Huhtanen and Hetta (2012). Moreover, 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 weighing of observations (St. Pierre, 2001; 2007). Thereby, and based on all that was discussed above, we don't see any reason for restricting the use of change-over designs in CSPF supplementation studies and 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. The reasons why fat supplements reduce DMI have been extensively discussed by Allen (2000). The most recent findings indicated that the hypophagic effect of CSPF seems to be associated with the secretagogue action of oleic acid on gut hormones and peptides related to satiety, such as cholecystokinin and glucagon-like peptide-1 respectively (Relling and Reynolds, 2007). de

Souza et al. (2018) reported that the inclusion of oleic acid in a whole cottonseed-based diet decreased the DMI of lactating dairy cows. Harvatine and Allen (2006) found that increasing unsaturated FA in dairy cow diets linearly decreased DMI by 15.1%. Previous meta-analyses reported that CSPF decreased DMI compared with CON by 0.64 (Rabbie et al., 2012) and 0.97 kg/d (Onetti and Grummer, 2004). Weld and Armentano (2017) reported that CSPF reduced DMI by 0.40 kg/d for each percentage unit of FA in the diet (%DM). We observed an overall reduction of 0.56 kg/d compared with CON, and a reduction of 0.22 kg/d for each unit of added CSPF in the diet DM. Presumably, these lower values observed in our study reflect the update of our data set, the differences in the criteria used to compose our data set (papers with ≤ 3% of fat inclusion), and the differences in statistical analysis.

Over the years, the negative effects of 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 oil (Czerkawski, et al., 1966; Ikwuegbuet and Sutton, 1982; Rufino et al., 2018; 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. Our results showed that CSPF increased NDF digestibility compared with CON by 1.60% units, with an increase of approximately 0.58% units for each unit of added CSPF in diet DM. The increase in NDF digestibility with CSPF can be associated with the decrease in DMI in this treatment, but this may not be the only determining cause as the reduction in DMI did not alter the digestibility of DM itself, or the digestibilities of the other nutrients. Indeed, when we tested DMI as covariate, CSPF still tented to increase NDF digestibility compared with CON, but the difference reduced (1.09% units; $P = 0.08$). Despite CSPF be satisfactorily stable at rumen pH, some dissociation occurs (Sukhija and Palmquist, 1990), so that part of C16:0 is exposed in the ruminal fluid. Palmitic

acid is an important component in the biomembrane of fibrolytic bacteria, and several studies have demonstrated that C16:0 supplementation increases NDF digestibility (Mackie et al., 1991; Vlaeminck et al., 2006; Piantoni et al., 2013; de Souza and Lock, 2019). Thereby, it is possible that CSPF increased NDF digestibility as a combination of both factors: the reduction of DMI, and the effect of C16:0 on fibrolytic bacteria. Furthermore, the dissociation of CSPF also allows that part of cis-9 C18:1 is free in the rumen, but when a fat supplement is included at ≤ 3% of diet DM, this does not seem to be a problem. de Souza et al (2018) supplemented cows with a blend of ~45% C16:0 and ~35% *cis*-9 C18:1 at 1.77% of diet DM, and found an increase in NDF digestibility by 0.8% units compared with CON.

Fatty acid supplementation has usually decreased total FA digestibility (Palmquist, 1991; Boerman et al., 2015). Despite the high proportion of FA in CSPF diets (5.08% diet DM), CSPF did not decrease FA digestibility, and displayed a numerical increase compared to CON (+2.68%; P = 0.12). This can be attributed to the unsaturated FA presented in CSPF (cis-9 C18:1 and C18:2), which have an additive effect to emulsifying properties of lysolecithin, mainly by increasing the micellar solubility of C18:0 (Freeman, 1969). Similarly, de Souza et al. (2018) reported that a diet with CSPF had the same FA digestibility as a CON diet, and additionally increased the digestibilities of 16-carbon, 18-carbon and total FA compared with a diet high in a mixed FA supplement (~40% C16:0 + ~40% C18:0). These findings allow us to hypothesize that FA profile reaching the duodenum can impact more FA digestibility than the total flow of FA in the small intestine (Boerman et al., 2015; Rico et al., 2017; de Souza et al., 2018). To better understand the mechanisms underlying the FA absorption and its limitations, more research is needed to examine the interactions between the amount and profile of FA reaching the duodenum.

Compared with CON, CSPF increased milk yield by 1.53 kg/d, with an increase of 0.42 kg/d for each unit of added CSPF in diet DM. Rabbie et al (2012) reported that CSPF

increased milk yield by 1.55 kg/d, and Onetti and Grummer (2004) reported 1.29 kg/d. Some mechanisms can explain the positive effect of CSPF on milk yield. Overall, 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; Palmquist, 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).

It is important to highlight that the production responses related to FA supplementation can vary depending on FA profile. As well, other nutritional aspects could explain the positive effect of CSPF on milk yield. In our study, CSPF increased NDF digestibility, and did not affect FA digestibility, possibly 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 the milk fat. In studies with grazing cows, CSPF also increased milk fat yield due to the increment of mixed and preformed milk FA (Batistel et al., 2017; de Souza et al., 2017). Other meta-analyses have reported that CSPF increased milk fat yield, although those were not able to identify the FA sources effectively involved in this increment (Onetti and Grummer, 2004; Rabbie et al., 2012).

Microbial protein is the most important protein source for lactating dairy cows, because it presents the closest chemical scores in relation to milk protein, particularly its balance for lysine and methionine (Santos et al., 1998). Protein synthesis by bacterial cells depends on the availability of RDP and fermentable carbohydrates (Nocek, 1988; NRC, 2001). In our data set, diet CP content was 18% regardless of treatment, and CP digestibility was not affected by the inclusion of CSPF (65.1% units; $P = 0.32$). Therefore, we suppose that RDP was not limited in CSPF diets. Furthermore, CSPF increased NDF digestibility, which could have counterbalanced the reduction in DMI, resulting in a microbial protein

synthesis sufficient to provide MP similar to CON treatment. This could justify why CSPF did not affect milk protein yield. On the other hand, when CSPF did not decrease DMI, and still increased NDF digestibility, milk protein yield of lactating dairy cows increased by 0.09 kg/d (de Souza et al., 2017).

Compared with CON, CSPF supplementation did not affect milk fat content and, at the same time, it decreased milk protein content. These effects could have been the consequence of milk component dilutions, since CSPF increased milk yield and milk fat yield, but had no effect on milk protein yield. Similar results were observed in previous studies (Batistel et al., 2017; de Souza et al., 2017; de Souza et al., 2018).

Furthermore, as a result of the responses obtained for milk yield and the yield of milk components, CSPF increased 3.5% FCM and the energy output for milk. The inclusion of CSPF did not affect BW, BW change and BCS, which reflected as no effect of CSPF on energy output for maintenance. Unfortunately, the current data set did not allow for a robust assessment of CSPF on energy output for 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, heat stress, etc. Batistel et al. (2017) and de Souza et al. (2017) reported that the inclusion of CSPF in a corn-based diet reduced BW change and energy partitioning for body reserves in early-lactation grazing cows. On the other hand, different results have been observed for CSPF replacing soybean hulls in mid-lactation cows housed in individual tie stalls (de Souza et al., 2018; de Souza et al., 2019). Further studies are needed to comprehend all related aspects between CSPF supplementation and the energetic metabolism of lactating dairy cows.

Although CSPF increased milk fat yield compared with CON, this treatment reduced the yield of de novo milk FA. Partial dissociation of CSPF occurs at rumen, releasing *cis*-9 C18:1 to rumen biohydrogenation, which results in the formation of a wide range of trans-

C18:1 FA, including *trans*-10 C18:1 (Sukhija and Palmquist, 1990; Mosley et al. 2002). Elevated *trans*-10 C18:1 in milk fat is associated with the reduction in de novo FA synthesis (Dórea and Armentano, 2017). However, Lock et al. (2007) clearly demonstrated that *trans*-10 C18:1 has no effect on the yield of de novo milk FA, suggesting that *trans*-10 C18:1 is not a bioactive isomer, but simply a marker for altered rumen biohydrogenation pathways related to *trans*-10, *cis*-12 conjugated linoleic acid (CLA). Despite that CSPF have C18:2 in their composition (8.34 g/100g), it is unlikely that *trans*-10, *cis*-12 CLA had some downregulation in our study. Even at very small doses, *trans*-10, *cis*-12 CLA is a very potent inhibitor of the secretion of all FA, reducing the mRNA abundance of genes that encode not only the enzymes involved in de novo synthesis, but also the enzymes involved in the uptake, transport and desaturation of FA, and the formation of triglycerides as well. All of this promotes an overall decrease in milk fat yield (Baumgard et al., 2002; Bauman and Griinari, 2003). Unfortunately, we didn't have enough data to evaluate the effects of CSPF on milk *trans*-FA. This was reported in only one study, where *trans*-10, *cis*-12 C18:2 in milk fat was not detected (de Souza and Lock, 2018a; Table S2, Supplemental). Therefore, de novo milk FA yield seems to have been reduced as a consequence of the increase in preformed milk FA, because de novo milk FA compete with exogenous long-chain FA to be incorporated into the glycerol-3phosphate backbone (Palmquist, 2006; He and Armentano, 2011; He et al., 2012).

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 (Jessen, 2002; Lindmark Månsson, 2008). Distribution of C16:0 is uniform between the positions sn-1 (44.1%) and sn-2 (45.2%) of the glycerol backbone. More than 50% of de novo milk FA from 8 to 14-carbons are also esterified at sn-2, while C18:1 is esterified at sn-1 (37.5%) and sn-3 positions (41.5%). Over 98% of C4:0 and 93% of C6:0 are added to sn-3 (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. The reduction in de novo milk FA yield esterified at sn-2 is also observed in studies with enriched C16:0 supplements. 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, Table S2, Supplemental). In our study, however, CSPF did not affect milk C4:0 and decreased milk C6:0 yield. It has been demonstrated that the enzyme diglyceride acyl transferase does not specify differently among the FA esterified at the sn-3 position (Marshall and Knudsen, 1979). The lack of acyl specificity may have favored the esterification of *cis*-9 C18:1 at sn-3 in our study, since it was available in a great amount. This decreased milk C6:0 yield but had no effect on milk C4:0 yield. Ruminants are the unique animals to incorporate 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 fluidity at body temperature (Barbano and Sherbon, 1980; Scrimgeour and Harwood, 2007). Also, the pathway to generate C4:0 can involve advantages such as the use of a more efficient primer (butyryl-CoA), and the independence of malononyl-CoA, sparing ATP and NADPH (Palmquist et al. 1969; Lin and Kumar, 1972; Smith et al., 1974).

2.5. Conclusion

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, increased the yields of milk, milk fat and 3.5% FCM, and decreased protein content. The increase in milk fat yield was driven by the increase in the yields of mixed and preformed milk FA.

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Table1. List of the studies used and their experimental designs.

			Design type	
Authors	Year	Reported design[1]	Continous	Change-over
Schneider et al.	1988	SRD/CBD	X	X
Kent and Arambel	1988	CRD	X	
Schauff and Clark	1989	LSD		X
Canale et al.	1990	LSD		X
Andrew et al.	1991	SRD		X
Sklan and Moallem	1991	CBD	X	
Erickson et al.	1992	CRD	X	
Sklan et al.	1992	CBD	X	
Holter et al.	1992	CRD	X	
Schauff et al.	1992	LSD		X
Schauff and Clark	1992	LSD		X
Spicer et al.	1993	CRD	X	
Wu et al.	1993	CBD	X	
Salfer et al.	1994	CRD	X	
Sklan et al.	1994	CBD	X	
Simas et al.	1995	CBD	X	
Harrison et al.	1995	CBD	X	
Beaulieu and Palmquist	1995	CRD	X	
Cervantes et al.	1996	CBD	X	
Rodriguez et al.	1997	LSD		X
Moallem et al.	1997	CBD	X	
Garcia-Bojalil et al.	1998	CRD	X	
Moallem et al.	1999	CBD	X	
Moallem et al.	2000	CBD	X	
Umphrey et al.	2001	CBD	X	
Weiss and Wyatt	2004	LSD		X
DeFrain et al.	2005	CBD	X	
Garnsworthy et al.	2008	CBD	X	
Moallem et al.	2010	CRD	X	
Rico et al.	2014	LSD		X
Stoffel et al.	2015	LSD		X
de Souza and Lock	2018a	LSD		X
Ylioja et al.	2018	LSD		X

[1;] LSD = Latin square design; SRD = single reversal design; CRD = completely randomized design; CBD = completely block design.

Table 2. Descriptive statistic of diet fat inclusion and fatty acid (FA) composition of FA supplement.

	CSPF[1]			
Item	Mean	Min	Max	SEM
Fat added in the diet,%	2.20	0.78	3.00	0.08
FA profile, g/100g				
C16:0	45.9	41.8	51.5	1.39
C18:0	3.83	0.69	5.00	0.66
C18:1	37.8	33.3	40.2	1.17
C18:2	8.34	7.40	9.50	0.36

[1]Calcium-salts of palm fatty acids.

Table 3. Descriptive statistic of nutrient composition of treatment diets.

	CON[1]				CSPF[2]			
Nutrient composition, % of DM	Mean	Min	Max	SEM	Mean	Min	Max	SEM
NDF	33.1	25.2	47.0	0.83	32.5	24.8	46.0	0.75
CP	18.0	15.2	22.0	0.30	17.9	15.6	21.4	0.26
Fatty acids	3.45	1.40	6.76	0.22	5.08	2.40	8.63	0.20

[1]Control; [2]Calcium-salts of palm fatty acids.

Table 4. Descriptive statistic of variables according with experimental design.

Itens	Change-Over						Continuous					
	N		Overall				n		Overall			
	CON[1]	CSPF[2]	Mean	Min	Max	SEM	CON[1]	CSPF[2]	Mean	Min	Max	SEM
DMI, kg/d	17	17	23.2	16.0	29.5	0.93	31	37	21.4	16.2	25.0	0.66
Nutrient digestibility,%												
DM	11	11	65.5	61.1	69.9	0.92	7	7	64.8	55.8	68.6	1.13
CP	7	7	65.5	59.7	70.2	2.59	6	6	64.6	54.7	73.2	2.47
NDF	11	11	45.0	31.2	57.2	3.38	9	9	47.1	36.5	61.7	4.01
Yield, kg/d												
Milk	18	18	34.6	22.5	48.9	1.67	29	35	34.9	23.6	42.9	1.21
Fat	18	18	1.26	0.89	1.78	0.07	28	34	1.19	0.79	1.71	0.05
Protein	17	17	1.04	0.82	1.47	0.05	27	33	1.07	0.76	1.42	0.03
3.5% FCM	18	18	33.4	27.2	48.5	0.98	23	29	33.2	24.0	43.8	0.76
ECM	19	19	33.8	21.2	48.3	1.12	22	28	33.6	24.3	42.9	0.73
Milk content, g/100g												
Fat	18	18	3.57	2.8	5.19	0.13	34	37	3.42	2.67	4.48	0.09
Protein	17	17	3.15	2.88	3.95	0.05	32	35	3.01	2.55	3.69	0.03
Energy output, Mcal/d												
Milk	17	17	24.6	19.2	33.5	1.27	28	31	23.1	16.6	28.2	0.81
Maintenance	8	8	9.23	11.1	9.60	0.26	15	19	8.78	11.4	9.64	0.21
Milk fatty acid yield, g/d												
De novo	8	8	293	182	417	32.8	7	9	255	190	327	36.8
Mixed	9	9	461	288	587	37.0	8	10	354	281	439	40.1
Preformed	9	9	360	284	628	88.5	8	10	429	230	673	96.7
Saturated	9	9	771	443	1.091	94.0	8	10	660	441	820	103
MUFA	9	9	258	196	423	57.2	8	10	270	133	465	61.9
PUFA	8	8	43.9	21.6	59.6	7.55	8	10	46.4	22.3	64.1	7.08
Milk fatty acid content, g/100g												
De novo	8	8	22.1	17.5	25.7	2.21	6	8	23.5	15.8	34.0	2.35
Mixed	9	9	33.5	25.4	47.3	2.57	7	9	32.4	29.3	36.8	2.85

Preformed	9	9	27.8	25.6	41.4	6.08	7	9	38.7	23.9	50.1	6.75
Saturated	9	9	57.2	28.6	80.3	6.68	7	9	60.4	43.2	70.9	7.38
MUFA	9	9	19.2	17.7	30.9	4.36	7	9	24.3	13.9	34.6	4.79
PUFA	8	8	3.54	1.95	4.69	0.52	7	9	4.17	2.38	5.93	0.57

[1]Control; [2]Calcium-salts of palm fatty acids.

Table 5. Descriptive statistic of the variables according with treatments diets.

	CON[1]					CSPF[2]				
Itens	n	Mean	Min	Max	SEM	n	Mean	Min	Max	SEM
DMI, kg/d	48	22.3	16.2	29.5	0.56	54	21.7	16.3	28.7	0.56
Nutrient Digestibility,%										
DM	18	65.2	55.8	69.9	0.73	18	65.3	59.1	69.0	0.73
CP	13	64.6	54.8	70.8	1.70	13	65.6	54.7	73.2	1.70
NDF	18	45.1	31.2	59.30	2.5	18	46.7	33.4	61.7	2.50
Total FA	13	68.9	53.5	88.1	3.26	13	71.6	57.1	90.0	3.26
Yield, kg/d										
Milk	47	34.0	22.5	48.3	1.01	53	35.6	23.6	48.9	1.00
Fat	46	1.19	0.82	1.71	0.04	52	1.24	0.79	1.78	0.04
Protein	44	1.06	0.76	1.47	0.03	50	1.06	0.77	1.46	0.03
3.5% FCM	41	33.9	24.6	47.5	1.14	47	35.2	24.0	48.5	1.14
ECM	41	33.7	21.2	47.6	1.20	47	34.8	22.4	48.3	1.19
Milk Content, g/100g										
Fat	52	3.45	2.67	5.19	0.08	55	3.49	2.68	5.19	0.08
Protein	49	3.08	2.58	3.95	0.03	52	3.03	2.55	3.69	0.03
BW, kg	23	599	530	745	16.7	27	591	526	742	16.6
BW change, kg/d	9	0.22	-0.07	0.61	0.08	13	0.16	-2.00	0.78	0.08
BCS	10	3.23	2.60	3.57	0.05	14	3.13	2.50	3.56	0.05
Energy output, Mcal/d										
Milk	43	23.0	17.0	32.6	0.70	46	23.9	16.6	33.5	0.70
Maintenance	23	9.64	8.83	11.4	0.16	27	9.59	8.78	11.4	0.16
Milk Fatty Acid Yield, g/d										
De novo	13	297	225	416	24.0	15	256	250	182	24.0
Mixed	15	404	281	587	32.7	17	417	283	580	32.7
Preformed	15	370	299	601	62.2	17	414	233	673	62.1
Saturated	15	727	440	1.09	68.6	17	71.5	453	1.09	68.4
MUFA	15	233	134	389	40.6	17	291	138	465	40.8
PUFA	14	43.9	21.6	64.1	4.95	16	46.6	22.3	62.5	4.93
Milk Fatty Acid Content, g/100g										
De novo	13	24.8	18.8	34.0	1.54	15	20.8	15.8	30.3	1.53
Mixed	15	32.3	25.4	47.3	1.82	17	33.6	27.7	46.2	1.82
Preformed	15	31.2	23.9	46.0	4.68	17	34.1	24.8	50.1	4.68
Saturated	15	59.4	28.6	80.3	4.73	17	58.0	31.5	75.8	4.73
MUFA	15	20.3	13.9	29.4	3.20	17	22.7	14.73	34.6	3.20
PUFA	14	3.79	1.95	5.83	0.38	16	3.88	1.99	5.93	0.38

[1]Control; [2]Calcium-salts of palm fatty acid.

Table 6. Effect of experimental designs and their interactions with treatments on DMI, nutrient digestibility, production responses, and energy output in lactating dairy cows.

	Mean difference		Variance[2]		*P*-value[3]		
Itens	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	Trt	Design	Trt×Design
DMI, kg/d	1.71	1.144	2.68	1.09	0.03	0.14	0.68
Nutrient digestibility, %							
DM	0.69	1.46	2.24	1.37	0.95	0.64	0.75
CP	0.85	3.58	5.01	2.32	0.34	0.82	0.67
NDF	-2.11	5.24	8.89	1.41	0.01	0.69	0.27
Total FA	3.28	3.28	9.39	3.99	0.27	0.65	0.12
Yield kg/d							
Milk	-0.30	2.07	4.56	2.85	0.02	0.88	0.90
3.5% FCM	0.02	0.25	5.22	1.33	0.04	0.63	0.85
ECM	0.02	0.25	5.55	1.34	0.07	0.90	0.89
Fat	0.07	0.08	0.17	0.10	0.02	0.42	0.26
Protein	-0.03	0.05	0.00	0.10	0.94	0.54	0.96
Concentration, g/100g							
Fat	0.15	0.16	0.30	0.22	0.43	0.35	0.59
Protein	0.14	0.06	0.10	0.10	0.01	0.02	0.20
Energy output, Mcal/d							
Milk	1.51	1.50	3.07	1.35	0.13	0.32	0.75
Maintenance	-0.03	0.33	0.46	0.22	0.52	0.92	0.67

[1] Difference between experimental designs (Change-over – Continuous).
[2] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.
[3] 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 7. Effect of experimental designs and their interactions with treatments on milk fatty acids.

	Mean difference		Variance[2]		*P*-value[3]		
	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	Trt	Design	Trt×Design
Summation[4], g/d							
De novo	38.2	49.3	2.00	0.45	<0.01	0.45	0.46
Mixed	107	54.6	2.46	0.48	0.03	0.06	0.41
Preformed	30.2	9.00	3.81	0.74	<0.01	0.74	0.32
Saturated	110	140	6.50	0.67	0.43	0.44	0.74
MUFA	50.7	62.8	2.54	0.72	<0.01	0.43	0.50
PUFA	-2.43	10.3	0.42	0.17	0.20	0.82	0.66
Summation[4], g/100g							
De novo	-1.41	3.23	3.99	1.64	<0.01	0.67	0.61
Mixed	1.10	3.86	5.62	0.50	0.06	0.79	0.86
Preformed	-4.06	5.43	7.46	1.01	<0.01	0.47	0.14
Saturated	-3.20	9.96	14.5	1.57	0.33	0.75	0.73
MUFA	-0.15	4.07	5.35	1.24	<0.01	0.97	0.46
PUFA	0.00	0.01	1.12	0.26	0.37	0.43	0.66

[1] Difference between experimental designs (Change-over – Continuous).
[2] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.
[3] 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.
[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).

Table 8. Effect of calcium salts of palm fatty acids on DMI and nutrient digestibility.

	Mean difference		Variance[2]		
	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	*P*-value
DMI, kg/d	-0.56	0.21	2.72	1.09	0.01
Nutrient digestibility, %					
DM	0.12	0.48	2.15	1.36	0.80
CP	0.91	0.89	4.55	2.28	0.32
NDF	1.60	0.57	8.54	1.43	0.01
Total FA	2.68	1.64	8.92	4.17	0.12

[1] Difference between treatments (Calcium salts of palm fatty acids – Control).
[2] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

Table 9. Effect of calcium salts of palm fatty acids on production responses.

	Mean difference		Variance[2]		
	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	*P*-value
Yield kg/d					
Milk	1.53	0.56	4.48	2.83	<0.01
3.5% FCM	1.28	0.60	5.12	1.32	0.04
ECM	1.12	0.60	5.43	1.32	0.07
Fat	0.04	0.02	0.17	0.10	0.04
Protein	0.00	0.02	0.10	0.10	0.94
Lactose	0.02	0.09	0.26	0.10	0.77
Concentration, g/100g					
Fat	0.03	0.04	0.30	0.22	0.43
Protein	-0.05	0.02	0.10	0.10	0.02
Lactose	0.02	0.02	0.10	0.00	0.34
BW, kg	-7.98	8.72	40.7	30.8	0.36
BW change, kg/d	-0.06	0.05	0.20	0.10	0.25
BCS	-0.10	0.06	0.00	0.14	0.15
Energy output, Mcal/d					
Milk	0.91	0.32	3.07	1.35	0.01
Maintenance	-0.05	0.06	0.42	0.22	0.45

[1] Difference between treatments (Calcium salts of palm fatty acids – Control).
[2] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.

Table 10. Effect of calcium salts of palm fatty acids on milk fatty acids.

	Mean difference		Variance[2]		
Itens	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	*P*-value
Yield, g/d					
De Novo	-41.1	5.34	1.95	0.45	<0.01
Mixed	13.4	5.31	2.99	0.47	0.01
Preformed	68.0	15.4	3.59	0.74	<0.01
Concentration, g/100g					
De Novo	-3.99	0.61	3.69	1.61	<0.01
Mixed	1.28	0.55	5.30	0.50	0.05
Preformed	4.04	0.80	7.24	1.00	<0.01

[1] Difference between treatments (Calcium salts of palm fatty acids – Control).
[2] $\hat{\sigma}_s$ = square root of the estimated study variance; $\hat{\sigma}_e$ = square root of the estimated residual variance.
[3] 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). Concentrations and yields of individual fatty acids are reported in Supplementary Table 2.

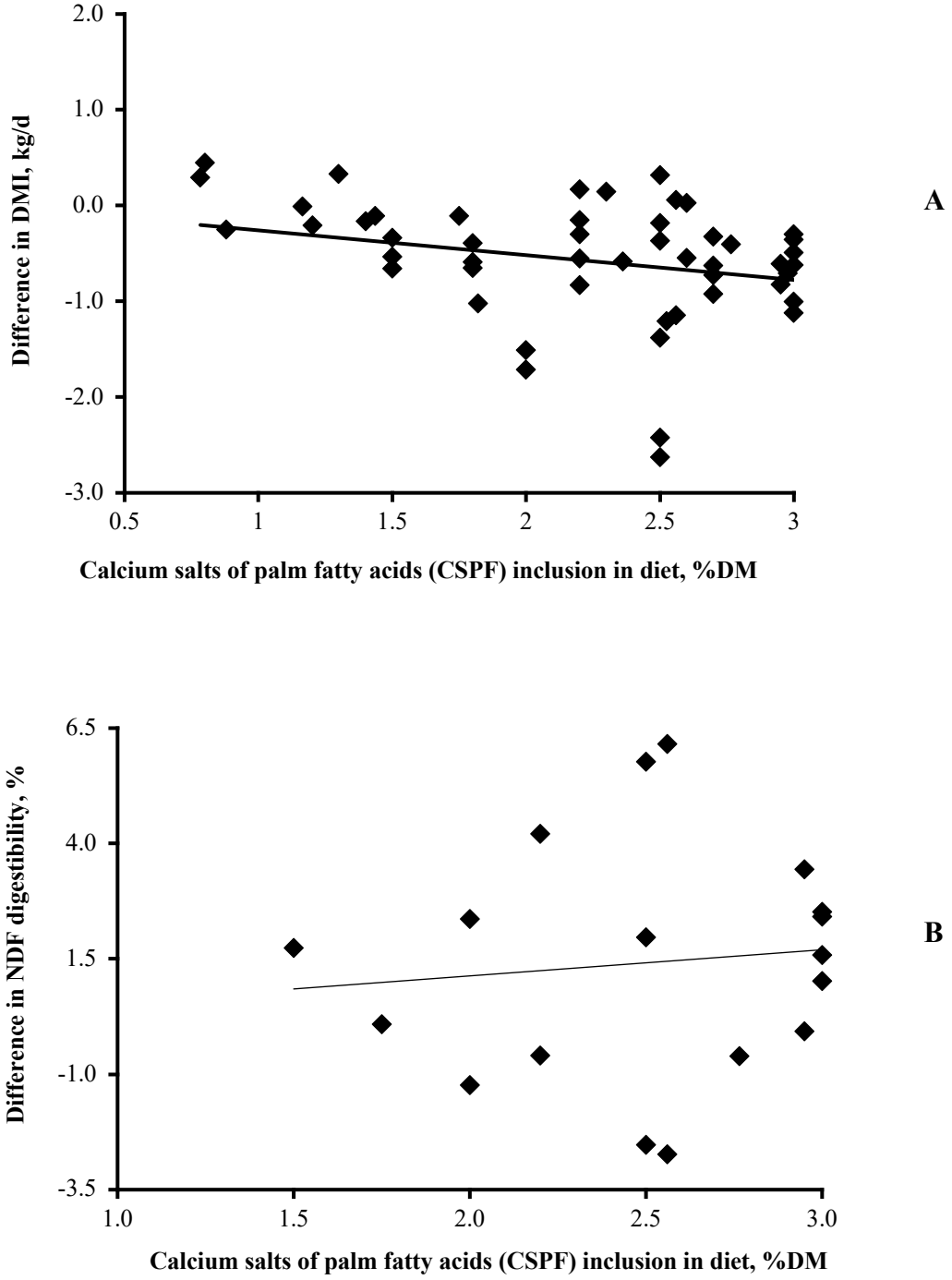


Figure 1. A: Difference in DMI (kg/d) = -0.22 (± 0.05) × CSPF inclusion in diet (%DM), $P < 0.01$; B**:** Difference in NDF digestibility = 0.58 (±0.24) × CSPF inclusion in diet (%DM), $P = 0.05$.

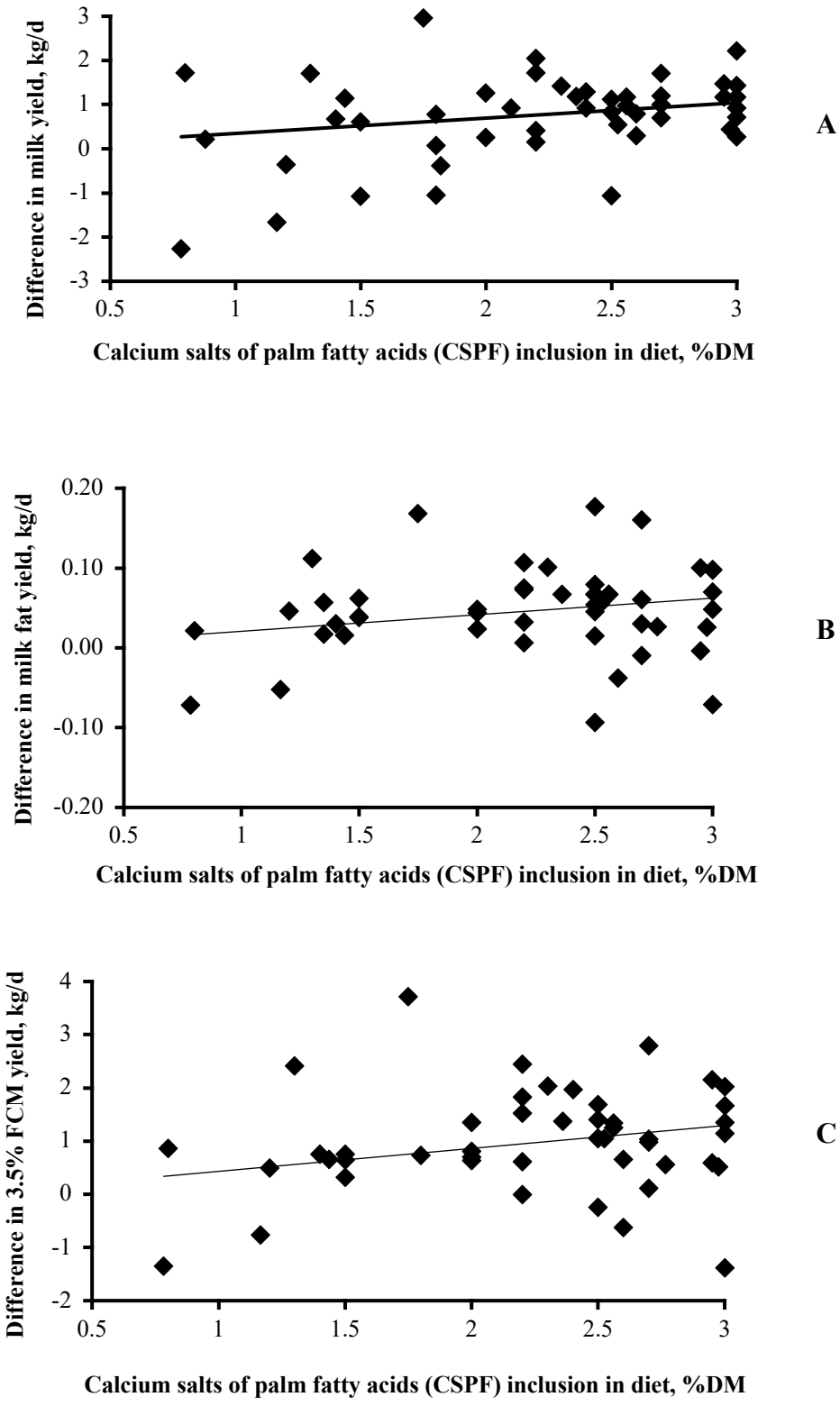


Figure 2. A: Difference in Milk yield (kg/d) = 0.42 kg/d (± 0.11) × CSPF inclusion in diet (%DM), $P < 0.01$; **B:** Difference in milk fat yield = 0.02 kg/d (±0.01) × CSPF inclusion in diet (%DM), $P < 0.01$; **C:** Difference in 3.5% FCM yield (kg/d) = 0.44 kg/d (± 0.20) × CSPF inclusion in diet (%DM), $P < 0.04$.

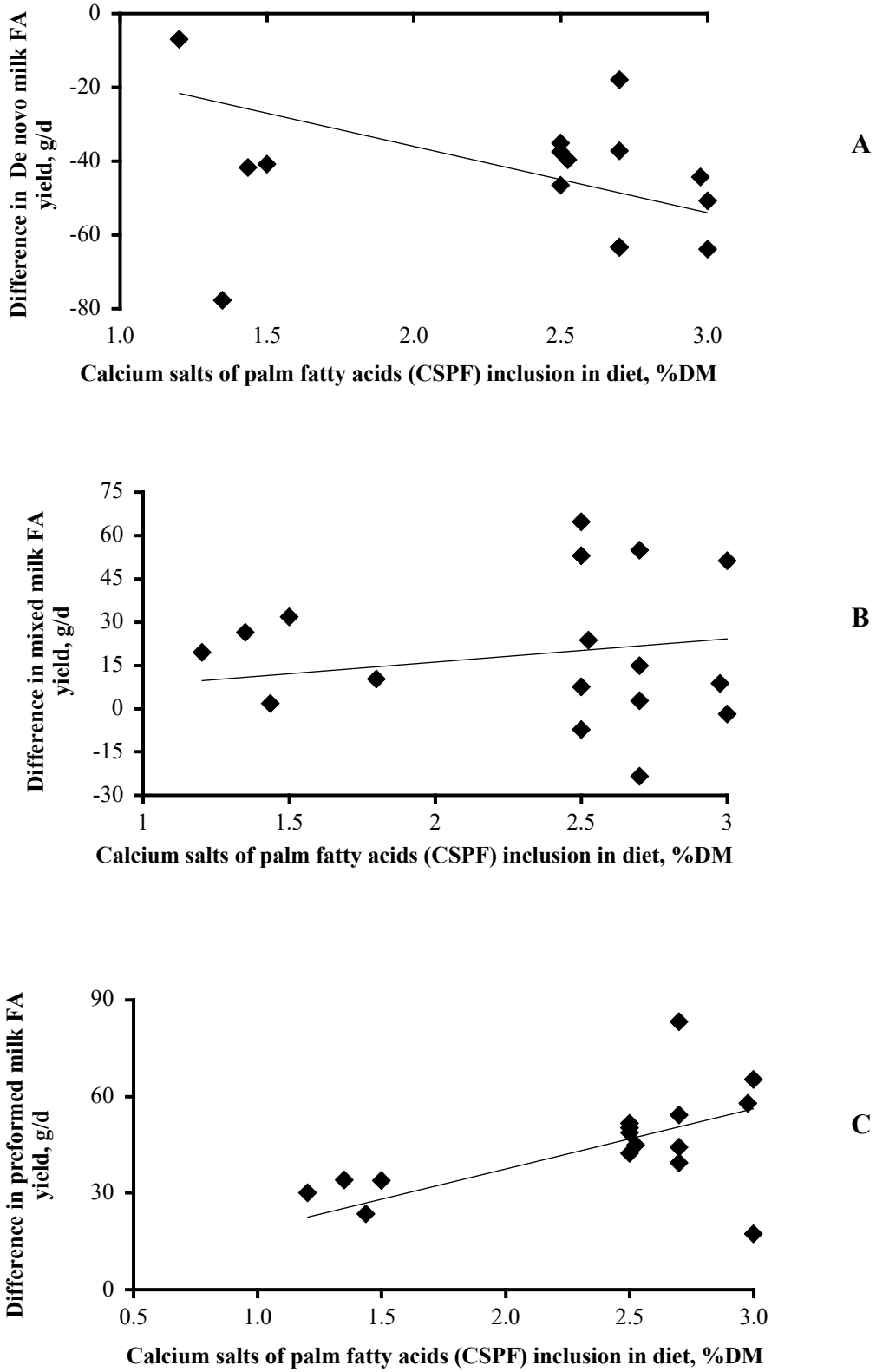


Figure 3. A: Difference in de novo milk FA yield (kg/d) = -18.4 kg/d (± 2.52) × CSPF inclusion in diet (%DM), $P < 0.01$; **B:** Difference in mixed milk FA yield = 6.80 kg/d (±1.17) × CSPF inclusion in diet (%DM), $P < 0.01$; **C:** Difference in preformed milk FA yield = 20.0 kg/d (±4.93) × CSPF inclusion in diet (%DM), $P < 0.01$.

Supplementary Table 1. Descriptive statistic of individual milk fatty acids according with treatments diets

	CON[1]					CSPF[2]				
	N	Mean	Min	Max	SEM	n	Mean	Min	Max	SEM
Milk FA Yield, g/d										
C4:0	9	40.2	28.7	55.1	4.78	11	42.6	30.9	60.6	4.77
C6:0	13	26.7	16.3	36.5	2.81	15	24.7	14.2	35.8	2.80
C8:0	13	16.5	11.3	21.7	1.51	15	14.1	7.80	19.2	1.50
C10:0	13	38.5	23.8	51.6	3.68	15	30.6	14.1	42.5	3.66
C12:0	13	44.6	29.7	58.5	3.64	15	34.3	18.2	47.2	3.62
C14:0	13	141	107	193	10.7	15	118	77.4	170	10.7
C14:1	8	13.1	10.3	15.9	0.68	8	10.1	8.51	11.7	0.68
C16:0	15	390	269	560	31.5	17	403	267	559	31.4
C16:1	11	22.2	19.1	26.8	1.37	11	21.3	18.5	25.2	1.37
C18:0	14	122	72.3	189	13.3	16	127	72.7	195	13.3
C18:1	11	247	166	359	25.0	11	313	235	437	25.0
C18:2	14	39.7	20.5	64.1	5.15	16	41.7	18.3	62.4	5.13
C18:3	7	6.99	2.88	13.8	1.59	9	7.24	3.89	12.9	1.59
Milk FA Content, g/100g										
C4:0	9	3.24	2.49	4.13	0.27	11	3.33	2.53	4.20	0.27
C6:0	13	2.19	1.37	3.16	0.22	15	2.03	1.15	2.79	0.21
C8:0	13	1.33	0.91	1.98	0.13	15	1.13	0.68	1.78	0.13
C10:0	13	3.07	2.09	5.01	0.31	15	2.38	1.36	4.30	0.31
C12:0	13	3.63	2.61	5.99	0.30	15	2.68	1.75	5.03	0.30
C14:0	13	11.6	9.41	13.9	0.55	15	9.62	7.45	12.7	0.55
C14:1	8	0.97	0.80	1.50	0.07	8	0.74	0.65	0.95	0.08
C16:0	15	31.3	23.6	45.6	1.80	17	32.3	25.7	44.6	1.79
C16:1	11	1.85	1.50	2.12	0.06	11	1.77	1.40	2.02	0.06
C18:0	14	10.0	7.24	17.8	0.76	16	10.2	7.04	16.7	0.75
C18:1	11	21.3	14.9	27.1	1.82	11	26.2	19.5	32.5	1.82
C18:2	14	3.38	1.95	5.83	0.45	16	3.39	1.95	5.93	0.46
C18:3	7	0.55	0.30	1.12	0.09	9	0.51	0.35	0.99	0.09

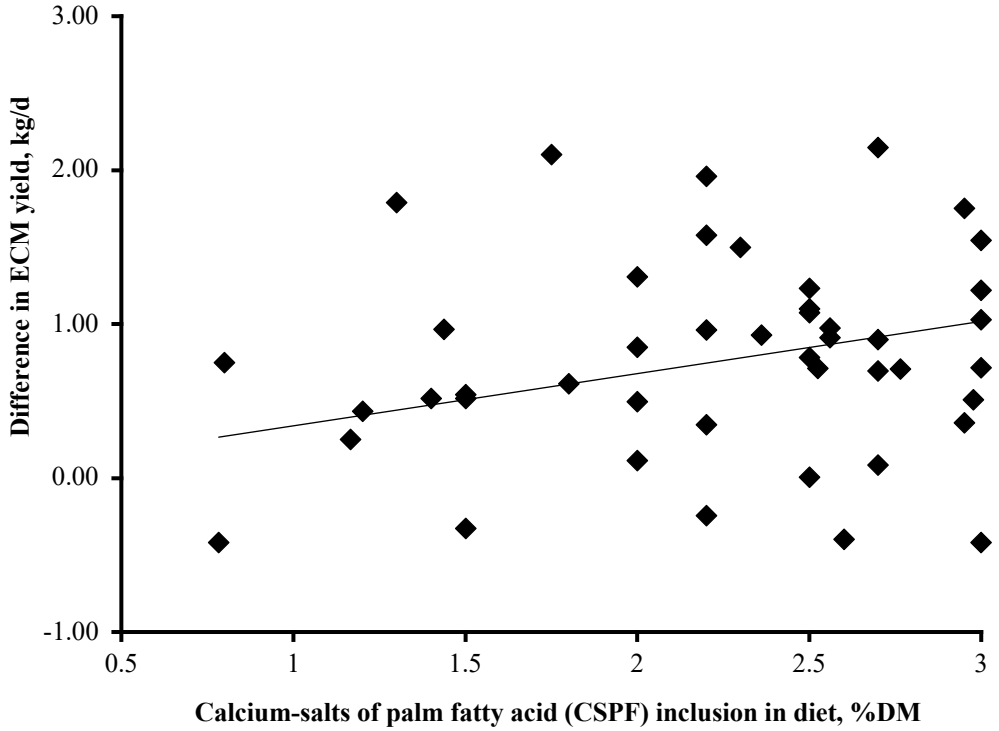
[1]Control; [2]Calcium-salts of palm fatty acids.

Supplementary Table 2. Effect of calcium salts of palm fatty acids on individual milk fatty acids.

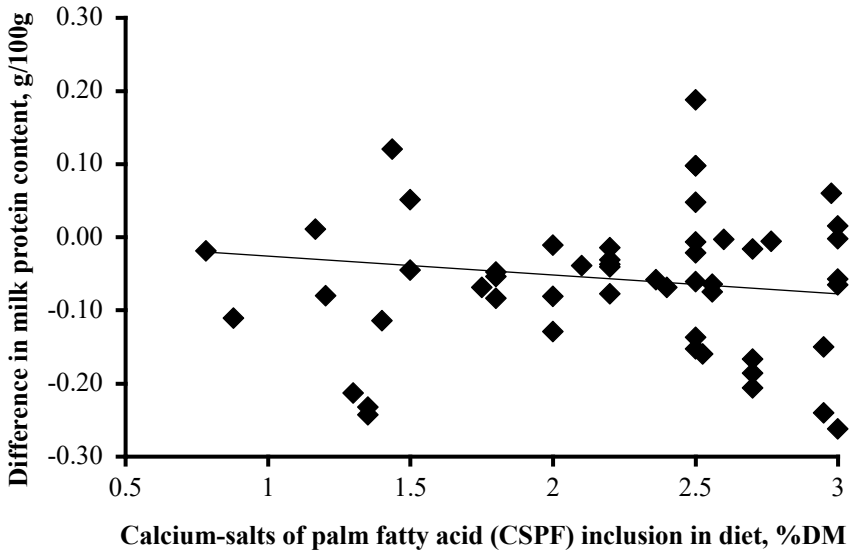
	Mean difference		Variance[2]		
Itens	Estimate[1]	SE	$\hat{\sigma}_s$	$\hat{\sigma}_e$	*P*-value
Milk FA Yield, g/d					
C4:0	2.42	2.42	0.10	0.01	0.12
C6:0	-2.03	0.79	0.05	0.00	0.02
C8:0	-2.49	0.57	13.3	2.31	<0.01
C10:0	-7.96	1.55	0.07	0.17	<0.01
C12:0	-10.2	1.70	0.07	0.02	<0.01
C14:0	-22.3	3.31	0.71	0.08	<0.01
C14:1	-3.07	0.95	0.00	3.67	<0.01
C16:0	13.7	5.23	8.26	0.21	0.02
C16:1	-0.84	1.06	0.01	0.01	0.44
C18:0	4.60	3.77	1.34	0.34	0.26
C18:1	66.0	17.8	1.93	1.75	<0.01
C18:2	2.08	1.90	0.18	0.03	0.29
C18:3	0.24	0.37	11.8	0.53	0.53
Saturated	-11.9	13.7	6.36	0.66	0.39
MUFA	55.5	14.8	2.50	0.71	<0.01
PUFA	-2.73	2.12	0.40	0.17	0.21
Milk FA Content, g/100g					
C4:0	0.08	0.03	0.35	0.01	0.03
C6:0	-0.17	0.04	0.27	0.01	<0.01
C8:0	-0.19	0.03	0.10	0.01	<0.01
C10:0	-0.69	0.12	0.52	0.11	<0.01
C12:0	-0.95	0.16	0.47	0.17	<0.01
C14:0	-2.06	0.28	1.75	0.36	<0.01
C14:1	-0.23	0.05	0.01	0.01	<0.01
C16:0	1.02	0.45	28.0	0.87	0.05
C16:1	-0.08	0.08	0.00	0.04	0.38
C18:0	0.13	0.30	4.07	0.32	0.66
C18:1	4.81	1.03	13.38	5.91	<0.01
C18:2	0.01	0.08	1.47	0.05	0.90
C18:3	-0.03	0.03	0.04	0.00	0.39
Saturated	-1.39	1.38	13.7	1.55	0.34
MUFA	3.68	0.80	4.97	1.22	<0.01
PUFA	0.0	0.09	1.10	0.26	0.39

[1] Difference between treatments (Calcium-salts of palm fatty acids – Control).

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



Supplementary Figure 1. Difference in ECM (kg/d) = 0.35 kg/d (±0.17) × CSPF inclusion in diet (%DM), $P = 0.06$.



Supplementary Figure 2. Difference in milk protein content (g/100g) = -0.02 g/100g (±0.01) × CSPF inclusion in diet (%DM), $P < 0.01$

3. CALCIUM SALTS OF PALM FATTY ACIDS AFFECT NUTRIENT DIGESTIBILITY, ENERGY PARTITIONING AND PRODUCTION RESPONSES OF MID-LACTATION GRAZING DAIRY COWS

INTERPRETATIVE SUMMARY

Calcium salts of palm fatty acids affect nutrient digestibility, energy partitioning and production responses of mid lactation grazing dairy cows. Our objective was to determine the effects of calcium salts of palm fatty acids (**FA**) on nutrient digestibility, energy partitioning, and production responses of mid lactation dairy cows grazing on tropical pastures. The treatments consisted of a supplemental concentrate without fat inclusion as control (**CON)**, and a supplemental concentrate with the inclusion of calcium salts of palm fatty acids (**CSPF**). Our results indicate that calcium salts of palm FA increase OM NDF, and FA digestibility, and alter energy partitioning, promoting increase in the yields of milk, milk fat, 3.5% FCM, and ECM.

ABSTRACT

The objective of our study was to determine the effects of calcium salts of palm fatty acids (**FA**) on nutrient digestibility, energy partitioning, and production responses of mid lactation dairy cows grazing tropical pastures. Cows were blocked by milk yield and BCS. All cows were kept in a rotational grazing system with elephantgrass (*Pennisetum purpureum* 'Cameroon'). The treatments were individually offered for 70-d and consisted of (1) supplemental concentrate as control (**CON**, 9.0 kg/d of concentrate without supplemental fat as fed basis); and (2) CON with calcium salts palm fatty acids (**CSPF**; 8.6 kg/d of CON + 0.4 kg/d of CSPF on a fed basis). Compared with CON, CSPF tended to decrease DMI, decreased the intakes of OM and NDF, and increased FA intake. Also, CSPF tended to increase DM digestibility, and increased the digestibilities of OM, NDF and FA. Compared with CON, CSPF decreased plasma insulin concentration, increased energy partitioning toward milk, and decreased energy partitioning toward body reserves. We observed an interaction between treatments and time for milk yield. Compared with CON, CSPF increased milk yield from 10 to 50-d (expect 30-d) of experiment, but there were no treatment differences from 60 to 70-d of experiment. Overall, CSPF increased milk yield by 1.60 kg/d compared with CON. There was no interaction between treatments and time for any other variables. Compared with CON, CSPF increased the yield of milk fat, 3.5% FCM, and ECM, and had no effect on the contents of milk fat, milk protein, milk lactose and MUN. There were no treatment differences for BW, BCS, and BCS change, but BW change tended to be greater for CON compared with CSPF. Compared with CON, CSPF decreased the yield of de novo milk FA, tended to increase the yield of mixed milk FA, and increased the yield of preformed milk FA. Feeding calcium salts of palm FA to mid lactation dairy cows grazing on tropical pasture increased OM, NDF and FA digestibility, and altered energy partitioning, promoting increases in the yields of milk, milk fat, 3.5% FCM, and ECM.

Keywords: Fat supplements, Grazing systems, Tropical pastures

3.1. Introduction

During the last two decades, management practices of tropical pastures have evolved significantly (da Silva et al., 2013). However, even well managed tropical pastures still impose limitations to energy intake and milk production of high genetic merit dairy cows and so, high energy supplements often need be fed (Batistel et al., 2017; de Souza et al., 2017). According to Schroeder et al. (2004), corn grain is the main concentrate supplement used for grazing dairy cows, but feeding supplemental fat has some advantages such as reduced risk of acidosis, and higher energy density. These authors also observed that DMI of grazing cows was generally similar or decreased with added fat, at the same time as increased milk and FCM, suggesting an improvement in energy efficiency. However, research with grazing cows fed supplemental fat are scarce, and based on overall evaluations that consider fatty acids (**FA**) from oilseeds and fat supplements with low digestibility (Weiss and Wyatt, 2004; Schroeder et al., 2004; de Souza et al., 2017).

Recent research has pointed out the importance of FA profile on digestibility, metabolism, and production responses of dairy cows (de Souza et al., 2018). Calcium salts of palm FA are one of the most common rumen inert fats used in dairy cows nutrition, being mainly composed of palmitic (C16:0; ~45%) and oleic (*cis*-9 C18:1; ~35%) acids (Loften and Cornelius, 2004; de Souza et al., 2019). With cows grazing in temperate pastures, Garnsworthy (1990) observed that replacing starch with calcium salts of palm FA increased milk fat content and yield, but tended to decrease milk protein content. Recent findings with early lactation dairy cows grazing on tropical pastures observed that calcium salts of palm FA was an effective strategy to alter energy partitioning, promoting increases in milk production, and yield of milk components (Batistel et al., 2017; de Souza et al., 2017). However, the aforementioned authors did not explore the hormonal and metabolic aspects related to fat

supplementation, as well as, to our knowledge, no studies were designed to evaluate the effects of calcium salts of palm FA in mid lactation dairy cows grazing on tropical pastures.

Therefore, the objective of our study was to determine the effects of calcium salts of palm FA on nutrient digestibility, energy partitioning, and production responses of mid lactation dairy cows grazing on tropical pastures. Our hypothesis was that supplementing calcium salts of palm FA would increase FA digestibility, energy partitioning to milk, and the yield of milk and milk fat of cows grazing on tropical pastures and fed a high corn supplement.

3.2. Materials and methods

3.2.1. Animal care

This study was conducted in Piracicaba, Sao Paulo, Brazil (22.7°S, 47.6°E and 546 m altitude) at the experimental farm of the University of Sao 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).

3.2.2. Design and Treatments

All animals received a common diet with no fat supplementation during a 15-d preliminary period to obtain baseline values. Twenty-two multiparous dairy cows (Jersey × Holstein), with (mean ± SEM) 477 ± 14.1 kg of BW and 144 ± 9.2 DIM, were used in a randomized complete block design, and were blocked by milk yield and BCS. All cows were kept in a grazing system. The treatments were offered for 70 d (treatment period), and consisted of (1) control (**CON**, 9.0 kg of concentrate without supplemental fat as fed basis); and (2) calcium salts palm FA (**CSPF**; 8.6 kg of CON + 0.4 kg of calcium salts of palm FA

cow/d as fed basis, Nutri Gordura Lac, Nutricorp Inc., Araras, Sao Paulo, Brazil). Treatments were fed individually, using a tie-stall facility in 2 equal feedings at 0500 and 1700 h before milking. The FA composition of calcium salts of palm FA, CON and pasture are showed in Table 1, the ingredients and nutrient composition of the pasture and concentrate supplements are showed in Table 2.

3.2.3. Grazing Management

All cows grazed elephant grass (*Pennisetum purpureum* L. Cameroon) pastures as one herd, in an area of 5.0 ha, divided in paddocks with 0.2 ha with free access to natural shade and fresh water. Elephant grass was managed in a rotational system based on a canopy height, with 100 cm being the entry height target. At a canopy height of 100 cm, this elephant grass cultivar has 95% 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). Post-grazing target corresponded to approximately 50% of the entry height target. The paddocks were fertilized with 60 kg of N/ha after each grazing. On average, cows were switched to a new paddock every day after evening milkings, and the average grazing interval was 22.4 ± 2.8 d. When the post-grazing height of the paddock was not reached, a group of dry cows was used to graze down the pasture to the target post-grazing height (50 cm).

3.2.4. Data and Sample Collection

Pre- and post-grazing heights were measured every day at 20 randomized points before animals entered and after they left the paddock respectively (Table 3). Pre- and post-grazing forage mass were measured in 2 paddocks every 10 d by clipping the forage inside a rectangular frame (0.94 m²) at 3 cm above ground level from sites that represented the mean sward height of the paddock. Total forage mass was weighed and 2 representative subsamples (500 g) 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 dried in a forced-air oven at 55°C for 72 h to determine the morphological composition of the forage mass (Table 3).

Forage and concentrate ingredients were collected for determination of their chemical composition every 10 d. Hand-plucked forage samples were taken at 20 randomized points before cows entered the paddock, by simulating the cows' grazing 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, Scientific, Philadelphia, PA).

Dry matter intake and nutrient digestibility were measured once during study through total fecal estimation from titanium dioxide (**TiO2**), and the estimation of indigestible NDF (**iNDF**) content of feces and feeds. For 15 d (from 35 to 49 d of experiment), cows received a daily dose of TiO2 (20 g/ cow per day), where half of total dose was received before each milking. In the last 5 d (44 – 49 d), fecal grab samples were collected after morning and afternoon milkings, and immediately stored at −20°C. Samples were subsequently thawed, dried at 55°C in a forced air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by cow.

Cows were milked twice a day at 0530 and 1730 h. Milk yield was recorded daily. Milk samples from both milkings were collected every 10 d and preserved with a bronopol preservative pill (Advanced Instruments, Norwood, MA). Milk samples used to determine milk FA profile were collected without preservative on 69 d of experiment, and stored at -20°C. Cows were weighed every 10 d after morning milking and scored for body condition 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).

Blood samples were collected in the last day of the experiment (70 d) by venipuncture of coccygeal vessels with a sterile needle and vacuum tubes (~15 mL), immediately before the cows have received the morning treatment (0500 h). The samples were stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Subsequently, plasma was transferred into microcentrifuge tubes and stored at −20°C.

3.2.5. Sample Analysis and Calculations

Forage intake was estimated from total fecal excretion and feed indigestibility. To estimate fecal excretion, fecal samples were analyzed for titanium concentration according to Myers et al. (2004). The iNDF contents of forage, concentrate and fecal samples (NDF remaining after 240 h of in situ incubation; Huhtanen et al., 1994) were determined to calculate indigestibility.

A Lucas test was used to estimate the FA digestibility of CSPF treatment. The mean intakes of FA and absorbed FA from cows fed the CON treatment were subtracted from the actual intakes of FA and absorbed FA from cows fed the CSPF treatment. This resulted in one observation per CSPF – CON pair for FA intake (Δintake) and absorbed FA (Δabsorbed). Thus, Δabsorbed was used as dependent variable (Y) and Δintake was used as independent variable (X). The resulting slope is assumed to be the FA digestibility of CSPF treatment (Weiss et al., 2011; de Souza et al., 2018).

Milk samples were analyzed for fat, protein, lactose, and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Milk component yields were calculated from milk component contents for each milking, and summed for a daily total. Yields of 3.5% fat-corrected milk (FCM) and energy-corrected milk (ECM) were calculated using the yields of milk and milk components as follow: 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)]; and ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (NRC, 2001).

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 GC (GC Shimatzu 2010 with automatic injection, Shimadzu Corporation), equipped with a SP-2560 capillary column (100 m × 0.25 mm i.d. with 0.02 μm film thickness, Supelco, Bellefonte, PA) as previously described by Marques et al. (2019).

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 [Σ < C16], mixed [Σ C16 + C16:1], and preformed [Σ >C16]), saturation (Σ SFA, Σ MUFA, and Σ PUFA) and odd linear chain (Σ odd linear FA). Odd-branched-chain FA were not used in the summation by source.

Mean daily BW change (kg/d) and BCS change were calculated for each cow during the experimental period by linear regression (Boerman et al., 2015a). Energy concentration of the diet was calculated based on nutrient digestibility (Boerman et al., 2015a) using equations (NRC, 2001) according to Harvatine and Allen (2006). Energy intake was calculated for each treatment from diet energy (DE, ME and NE_L) × DMI. Energy output (Mcal/d) for milk, body reserves, and grazing activities were calculated according to NRC (2001) as: 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 BCS was the average BCS generated by the linear regressions for each cow during the study and ΔBW was BW change; and Grazing activities output (Mcal/d) = (Δd × 0.0004 × BW) + (0.0012 × BW), where Δd was the average distance of 0.4 km between pasture and milking center. Energy output for maintenance was calculated by difference as follow: Energy output for maintenance (Mcal/d) = NE_L intake (Mcal/d) − Milk energy output (Mcal/d) − Body reserves output (Mcal/d) – Grazing activities output (Mcal/d). Energy partitioning was calculated as

the percentage of NE_L intake allocated to milk, body reserves, grazing activities, and maintenance (Boerman et al., 2015a).

Plasma concentrations of insulin, non-esterified fatty acids (NEFA), ß-hydroxybutyrate (BHB), gamma glutamyl transferase (GGT), total protein, BUN, albumin, and creatine kinase were determined using a Multiskam MS ELISA reader (Labsystems, Helsinki, Finland), using enzyme-linked immunoassay (ELISA) methods.

3.2.6. Statistical analysis

All data were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Production responses were analyzed with repeated measures according to the following model:

$$Yijkl = \mu + Fi + Tj + Bk + Cl\ (Bk) + Fi \times Tj + eijkl,$$

where Yijkl = the dependent variable, μ = the overall mean, Fi = the fixed effect of treatment, Tj = the fixed effect of time, Bk = the random effect of block, Cl (Bk) = the random effect of cow nested in block, Fi × Tj = the fixed effect of interaction between treatment and time, and eijkl = the residual error. The covariance structure used was selected based on the lowest Akaike's information criterion. Variables determined once (nutrient intake and digestibility, BW change, BCS change, energy output and partitioning, plasma hormones and metabolites, and milk FA profile) were analyzed without repeated measures, and without time and its interactions in the model. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Significant differences were declared at $P \leq 0.05$, and tendencies at $P \leq 0.10$ for main effects and interactions.

3.3. RESULTS

3.3.1. Nutrient Intake and Total-tract Digestibility

Compared with CON, CSPF tended to decrease DMI ($P = 0.09$) and CP intake ($P = 0.10$), decreased the intakes of OM ($P = 0.05$) and NDF ($P = 0.04$), and increased FA intake ($P < 0.01$, Table 4). Also, CSPF tended to increase DM digestibility ($P = 0.08$), increased the digestibilities of OM ($P = 0.04$), NDF ($P = 0.03$) and FA ($P = 0.05$), but had no effect on CP digestibility ($P = 024$, Table 4). By using a Lucas test, we observed that CSPF had a FA digestibility of 85%, and there was a positive linear relationship between the intake of supplemental FA by supplemental FA absorbed ($P < 0.01$, Figure 2).

3.3.2. Energy Intake, Energy Output, and Energy Partitioning

Compared with CON, CSPF increased diet NE_L by 0.10 Mcal/kg ($P = 0.01$), but had no effect on NE_L intake ($P = 0.75$, Table 5). Also, CSPF tended to increase energy output for milk ($P = 0.07$), decreased energy output for body reserves ($P = 0.01$) and maintenance ($P = 0.04$), and had no effect on energy output for grazing activities ($P = 0.21$, Table 5). When the energy output for maintenance was estimated without considering the discount from grazing activities, the differences were observed (CON = 11.4 Mcal/d, CSPF = 9.67 Mcal/d, SEM = 0.63, $P < 0.04$). For energy partitioning (as % energy intake), CSPF increased energy partitioning toward milk by 7.8% units ($P = 0.01$), decreased energy partitioning toward body reserves by 0.91% units ($P < 0.01$), and decreased energy partitioning toward maintenance by 8.1% units ($P < 0.01$, Table 5), compared with CON. There were treatment differences for energy partitioning toward grazing activities ($P = 0.44$, Table 5). When energy partitioning for maintenance was estimated without considering the discount from grazing activities, treatment differences were also observed (CON = 39.2%, CSPF = 31.6%, SEM = 2.35, $P < 0.01$).

3.3.3. Blood Hormones and Metabolites

Plasma concentrations of hormones and metabolites are shown in Table 6. We observed that CSPF decreased insulin ($P < 0.01$) and GGT ($P = 0.01$), increased NEFA ($P < 0.01$), but had no effect on BHB ($P = 0.64$, Table 6) compared with CON. We did not observe treatment differences for total protein ($P = 0.27$), BUN ($P = 0.23$), albumin ($P = 0.27$), and creatine kinase ($P = 0.82$, data not shown).

3.3.4. Production Responses

Overall, CSPF increased milk yield by 1.60 kg/d compared with CON ($P = 0.03$, Table 7). However, we observed an interaction between treatments and time for milk yield ($P < 0.01$, Table 7). Compared with CON, CSPF increased milk yield from 10 to 50-d (except d 30) of experiment ($P \leq 0.05$, Figure 1). There were no treatment differences or tendencies from 60 to 70-d of experiment ($P > 0.10$, Figure 1).

We did not observe interaction between treatments and time for any other variables ($P > 0.10$, Table 7). Overall, compared with CON, CSPF increased the yield of milk fat ($P = 0.04$), 3.5% FCM ($P = 0.05$) and ECM ($P = 0.04$), had no effect on yields of milk protein ($P = 0.30$) and lactose ($P = 0.25$), or on the contents of milk fat ($P = 0.62$), milk protein ($P = 0.37$), milk lactose ($P = 0.14$) and MUN ($P = 0.47$, Table 7). There were no treatment differences for BW ($P = 0.28$), BCS ($P = 0.13$), and BCS change ($P = 0.84$), but BW change ($P = 0.08$, Table 7) tended to be greater for CON compared with CSPF.

3.3.5. Milk Fatty Acid Concentration and Yield

Milk FA are derived from 2 sources: < 16 carbon FA from de novo synthesis in the mammary gland and > 16 carbon FA originating from extraction from plasma. Mixed source FA (C16:0 and *cis*-9 C16:1) originate from de novo synthesis in the mammary gland and

extraction from plasma. Compared with CON, CSPF decreased the yield of de novo milk FA ($P < 0.01$), tended to increase the yield of mixed milk FA ($P = 0.06$), and increased the yield of preformed milk FA ($P = 0.03$, Table 8).

We did not observe differences between treatments for the yield of milk SFA ($P = 0.36$), however, compared with CON, CSPF increased the yields of milk MUFA ($P = 0.03$), and milk PUFA ($P < 0.01$, Table 8), and reduced yield of odd linear milk FA ($P < 0.01$, Supplementary Table 1).

Compared with CON, CSPF had no effect on the yield of milk C4:0 ($P = 0.62$), decreased the yields of milk C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C15:0, and C17:0 ($P < 0.01$), as well as increased the yields of C16:0, *cis*-9 C18:1 ($P = 0.02$), total *trans*-C18:1; *cis*-9, *cis*-12 C18:2, and *cis*-9, *trans*-11 C18:2 ($P < 0.01$, Supplementary Table 1). Results for milk FA concentrations followed a similar pattern to those described for FA yields (Table 8, Supplementary Table 2).

3.4. DISCUSSION

There is limited information examines the impact of fat supplementation for grazing dairy cows, especially for animals grazing on tropical pastures. Previous studies have demonstrated the positive effects of calcium salts of palm FA on production responses of early lactation grazing dairy cows (Garnsworthy, 1990; Batistel et al., 2017; de Souza et al., 2017). However, to our knowledge, the impact of calcium salts of palm FA have not been studied in mid-lactation dairy cows grazing on tropical pastures. Therefore, the objective of our study was to determine the effects of supplementing calcium salts of palm FA on nutrient digestibility, energy partitioning, and production responses of mid lactation dairy cows grazing on tropical pastures and fed a high corn concentrate.

We observed that CSPF tended to decrease DMI and decreased OM intake. This discrepancy occurred due to the higher content of ash in the CSPF diet compared with the CON diet. The mechanisms that promote a reduction in intake by fat supplements may be associated with diet palatability, release of gut hormones and peptides, and an increase of FA oxidation in the liver (Allen, 2000). In our study, the treatments were offered daily in the same amount (9 kg/d), and orts were not observed. In the CSPF treatment, the reduction in OM intake was driven by a reduction in herbage intake of 1.19 kg/d (P = 0.04, data not shown). Thus, the decrease in OM intake clearly wasn't influenced by fat supplement palatability. It is also unlikely that this was mainly mediated by an increase of FA oxidation in the liver. While we observed that CSPF increased plasma concentration of NEFA, this treatment had no effect on BHB, indicating no FA oxidation compared with CON. When the cows are not in negative energy balance, normally the liver oxidation of NEFA is low (Emery et al., 1992; Palmquist, 1994). The hypophagic effect of CSPF is likely associated with the secretagogue action of unsaturated FA (mainly C18:1) on the release of cholecystokinin and glucagon-like peptide-1, reducing gastric emptying and slowing passage rate from the rumen (Allen, 2000; Relling and Reynolds, 2007; Bradford et al., 2008). In a meta-analysis by Rabbie et al. (2012), it is reported that calcium salts of palm FA decreased DMI compared to a non-fat supplemented control diet by 0.64 kg/d. de Souza et al. (2018) also reported that increasing levels of *cis*-9 C18:1 decreased DMI compared with non-fat supplemented control diet in the whole cottonseed basal diet, however, there was no effect in the soyhull basal diet. Interesting, calcium salts of palm FA had no effect on DM and OM intake of early lactation grazing dairy cows (Batistel et al., 2017; de Souza et al., 2017). Further studies are needed to more fully understand the impact of supplementation with calcium salts of palm FA and its interactions with feeding systems, dietary ingredients, and lactation phase.

A review by Loften and Cornellius (2004) indicated that the low inclusion of calcium salts of large chain FA in the diet of dairy cows is sufficient to depress intake to a level that would also reduce daily NE_L intake. These authors, however, did not differentiate calcium salts of palm FA from calcium salts of soybean FA. Although we observed that CSPF tended to reduce DMI and reduced OM intake, we observed that NE_L intake wasn't different between treatments, which was mainly mediated by the increase of diet NE_L content in CSPF compared with CON.

There is still a general idea that fat supplementation reduces NDF digestibility (Devendra and Lewis, 1974; Jenkins and Palmquist, 1984). Nonetheless, this concept has been built up on the findings of dietetic inclusion of pure oil and very high fat supplementation (Czerkawski, et al., 1966; Ikwuegbuet and Sutton, 1982). Conversely, we observed that CSPF increased NDF digestibility compared with CON. This is likely related with the effect that CSPF had to decrease NDF intake. de Souza et al. (2018) observed that a supplement blend of 45% of C16:0 + 35% of C18:1 *cis*-9 increased NDF digestibility, at the same time as decreased DMI compared with a non-fat supplemented control diet.

In addition, C16:0 in the rumen can potentially favor fibrolytic bacterial growth. Supplemental C16:0 could be incorporated into bacterial membranes, sparing ATP that would be used to synthesize de novo C16:0 (Vlaeminck et al., 2006; Hackmann and Firkins, 2015; de Souza et al., 2018). Indeed, several studies have consistently shown the positives effects of C16:0 enriched supplements on NDF digestibility of dairy cows (Piantoni et al., 2013; de Souza and Lock, 2018; de Souza and Lock, 2019). In a recent meta-analysis, Weld and Armentano (2017) observed that supplementation with calcium salts of palm FA had no effect on NDF digestibility of dairy cows in housed systems receiving TMR. On the other hand, other studies have demonstrated that CSPF supplementation increases NDF digestibility (Onetti and Grummer, 2004; de Souza and Lock, 2018). An increase in NDF digestibility with

the inclusion of calcium salts of palm FA has been also reported in grazing dairy cows (de Souza et al., 2017).

Digestibility of FA often decreases as supplemental fat is added in the diet (Palmquist, 1991). In recent a meta-analysis, Boerman et al. (2015b) observed that the increase in total FA duodenal flow decreases FA digestibility. However, we observed that CSPF increased FA intake, and also increased FA digestibility compared with CON. This was due the linear effect that CSPF had on FA absorption, as we demonstrated by the Lucas test. Similarly, Batistel et al. (2017) reported that feeding calcium salts of palm FA increased FA digestibility of grazing dairy cows by 5.2% units compared with non-fat control diet. These results are likely due the unsaturated FA presented in calcium salts of palm FA (*cis*-9 C18:1 and C18:2), which act as emulsifiers by increasing the micellar solubility of C18:0. Also, unsaturated FA are esterified faster, as well as require a smaller length of intestine for absorption than saturated FA (Freeman, 1969; Ockner et al., 1972). Among the unsaturated FA, C18:1 seems to have the highest intestinal digestibility (Doreau and Ferlay, 1994; Boerman et al., 2015b). de Souza et al. (2019) observed that increasing dietary *cis*-9 C18:1 linearly increased FA digestibility of dairy cows. Our results and these other findings support the idea that FA digestibility is more impacted by the profile of FA reaching the duodenum than the total flow of FA in the small intestine, up to a certain level (Rico et al., 2017; de Souza et al., 2018).

We observed that CSPF increased energy partitioning toward milk, and decreased energy partitioning toward body reserves compared with CON. This is likely associated with the role of insulin in modulating energy partitioning. Low plasma insulin concentrations decrease nutrient uptake by muscle and adipose tissue and increase nutrient uptake by the mammary gland, which is not insulin-responsive (Bauman and Elliot, 1983).

Indeed, CSPF decreased plasma insulin concentration, resulting in a consistent increase in milk fat yield, 3.5% FCM, and ECM, a tendency to reduce BW change, and an increase in NEFA concentration compared with CON. Our results agree with previous studies that evaluated lipogenic diets for lactating dairy cows. Garnsworthy (1990) observed that feeding grazing cows with calcium salts of palm FA resulted in greater outputs of milk energy, and reduced BW change compared to a starch based supplement. Boerman et al. (2015a) reported that partly replacing dietary corn with fiber and an enriched C16:0 supplement decreased insulin and energy partitioning toward body reserves, and increased energy partitioning toward milk. Similar results were observed by replacing starch with calcium salts of palm FA (van Knegsel et al. 2007a, 2007b; Batistel et al., 2017; de Souza et al., 2017). Conversely, de Souza et al. (2018) observed that the inclusion of calcium salts of palm FA replacing soyhulls increased insulin and promoted greater energy partitioning toward body reserves. The authors attributed these responses to the effect of *cis-9* C18:1 on stimulating insulin secretion from pancreatic β-cells (Itoh et al., 2003; Fujiwara et al., 2005). Further examination is needed to comprehend all related aspects between FA supplementation and its relations with the substitution of the ingredients in diet on the mode of action of insulin and energy partitioning.

Our results demonstrated that CSPF reduced energy use for maintenance, and energy partitioning toward maintenance compared with CON. We also observed that CSPF decreased plasma concentration of GGT, potentially indicating lower hepatic activity (Rodriguez-Jimenez, 2018). The liver of a dairy cow has several metabolic functions, and gluconeogenesis represents the main energy expenditure, corresponding to around 31% of total heat production of this organ (Baldwin, 1995; Reynolds, 2002). In CSPF treatment, calcium salts of palm FA partly replaced a supplement composed of 80% corn, thereby decreasing propionate and possibly gluconeogenesis. Although we did not collect ruminal

fluid, we observed that CSPF reduced the yield of milk odd linear-FA, a group of FA primarily synthesized from propionate by both ruminal microorganisms and the mammary gland (Vlaeminck et al., 2006). Furthermore, if the CSPF treatment has increased triglyceride synthesis (TG synthesis) in the liver from blood NEFA uptake, this activity expends 4.5% less energy than gluconeogenesis from propionate (Baldwin, 1995). Nonetheless, the results reported by van Knegsel et al. (2007b) demonstrated that FA supplementation did not alter TG synthesis compared with a non-fat control diet, and the NEFA increment was directed to milk fat yield. It is also important to highlight that the cows of our study were under heat-stress typically observed in tropical grazing, so that the incorporation of energy from FA without substantially increasing the heat increment may have favored the responses obtained for maintenance in CSPF. Similarly, Wang et al. (2010) demonstrated that replacing fermentable carbohydrates with supplemental fat decreased the body temperatures of heat stressed, plus saving a remarkable amount of metabolic heat.

We observed that CSPF increased milk yield by 1.60 kg/d compared with CON. Previous meta-analyses with dairy cows in housed systems reported that calcium salts of palm FA increased milk yield compared with non-fat control diets by an average of 1.4 kg/d (Onetti and Grummer, 2004; Rabbie et al., 2012). Recent studies with early lactation grazing dairy cows have reported that replacing ground corn with calcium salts of palm FA increased milk yield by an average of 4.25 kg/d (Batistel et al., 2017; de Souza et al., 2017). de Souza et al. (2018) observed that 1.5% of FA supplement blend (45% of C16:0 + 35% of C18:1 *cis*-9) increased milk yield by 1.9 kg/d compared with non-fat supplemented control diet. Overall, FA supplementation increases milk yield by promoting nutrient partition toward milk production, and by sparing energy by decreasing de novo milk FA synthesis (Bauman and Davis, 1974; Palmquist, 1994; Palmquist, 2006). We observed both aforementioned

mechanisms in our study, so that the higher milk fat yield found in CSPF was driven by the incorporation of milk C16:0 and preformed milk FA.

Among the reasons for the reduction of de novo milk FA synthesis, it is reported that calcium salts of palm FA provide a higher load of unsaturated FA in the diet, which likely overcome normal rumen biohydrogenation capacity (de Souza et al, 2019). We did not detect levels of *trans*-10, *cis*-12 C18:2 in milk fat of our samples, but it is important to consider that other FA produced as intermediates in rumen biohydrogenation have been shown to reduce milk fat (Bauman et al., 2011). On the other hand, normally the bioactive isomers from altered rumen biohydrogenation pathways are very potent inhibitors of the secretion of all FA, promoting an overall reduction in the yield of milk fat, not only in the yield of de novo milk FA (Baumgard et al., 2002; Bauman and Griinari, 2003). Thus, it is possible that CSPF reduced de novo milk FA yield as a consequence of the dietary increase in the exogenous long chain-FA, which compete to be incorporated onto the glycerol-3phosphate backbone (Palmquist, 2006; Glasser et al., 2008; He and Armentano, 2011; He et al., 2012).

We observed that CSPF decreased the yield of milk FA from 6 to 14-carbons, but had no effect on milk C4:0. There is a high specificity in the positions of FA along the glycerol backbone during milk triglyceride synthesis (Lindmark Månsson, 2008; Jessen, 2002). The reduction in the yield of milk FA from 8 to 14-carbons can be related with the increase in the uptake of milk C16:0 at sn-2 position of the glycerol backbone. Polyunsaturated FA are also predominantly esterified at sn-2 position (Jensen, 2002), and we found that C18:2 yield increased with CSPF. The esterification of *cis*-9 C18:1 at sn-3 may have decreased milk C6:0 yield. Over 98% of milk C4:0 is added to sn-3 (Jessen, 2002). However, decreases in the yield of milk C4:0 are often not observed, because of the low melting point (-5.3 °C) of this FA, which is important to maintain milk fluidity at body temperature (Barbano and Sherbon, 1980; Scrimgeour and Harwood, 2007). Likewise, the

pathway to generate C4:0 involves the use of a very efficient primer (butyryl-CoA), in an independent way from malonyl-CoA, which spares ATP and NADPH (Palmquist et al. 1969; Lin and Kumar, 1972; Smith et al., 1974). Similar milk FA composition has been previously reported in studies with calcium salts of palm FA (Batistel et al., 2017; de Souza et al., 2017 de Souza et al., 2018).

3.5. Conclusion

Feeding calcium salts of palm FA to mid lactation dairy cows grazing on tropical pasture increased OM, NDF and FA digestibility, and altered energy partitioning, promoting increases in the yields of milk, milk fat, 3.5% FCM, and ECM. The increase in milk fat yield was driven by the increase in the yields of C16:0 and preformed milk FA.

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Table 1. Fatty acid (FA) composition supplements of concentrate and forage fed during treatment period[1].

	Fat supplement[2]	Concentrate[3]	Forage[4]
FA, %	75.6	2.76	3.63
FA profile of each treatment, g/100g FA			
C12:0	3.70	0.19	0.82
C14:0	1.66	0.19	0.14
C16:0	43.2	23.6	20.2
C18:0	4.15	3.68	2.06
cis-9 C18:1	37.4	37.8	0.00
cis-9, *cis*-12 C18:2	7.31	33.5	10.0
cis-9, *cis*-12, *cis*-15 C18:3	0.23	0.45	50.3

[1] Average (n=7) composition of FA based on samples taken during the collection period.
[2] Calcium salts of palm fatty acids; Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil.
[3] Ground corn, soybean meal, mineral and vitamin premix (Nutrient composition in Table 2).
[4] Elephant grass (*Pennisetum purpureum* L. Cameroon; nutrient composition in Table 2).

Table 2. Ingredient and nutrient composition of forage and treatments.

Item	Forage[1]	Treatments	
		CON	CSPF
Ingredient, % DM			
Ground Corn		80.0	76.1
Soybean meal		15.0	14.3
Mineral and Vitamin mix[3]		5.00	4.76
Fat supplement[4]		0.00	4.82
Nutrient Composition, % DM			
OM	88.8	91.4	90.6
NDF	58.1	15.8	15.0
ADF	31.1	3.37	3.20
Lignin	3.22	0.12	0.11
CP	21.1	13.6	12.9
Ash	10.5	8.54	9.30
FA	3.63	2.76	5.65
12:0	0.03	0.01	0.12
14:0	0.01	0.01	0.06
16:0	0.73	0.62	1.93
18:0	0.07	0.10	0.22
cis-9 18:1	0.00	1.00	2.12
cis-9, *cis*-12 18:2	0.36	0.88	1.10
cis-9, *cis*-12, *cis*-15 18:3	1.83	0.01	0.02

[1] Elephant grass (*Pennisetum purpureum* L. Cameroon)
[2] Treatments were: 1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[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 D3, 1,250 of vitamin E.
[4] Calcium salts of palm fatty acids; Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil.

Table 3. Pasture characteristics at pre- and post-grazing.

	Pre-grazing	SD	Post-grazing	SD
Sward height (cm)	96.6	2.23	54.8	2.75
Forage mass (kg of DM/ha)	8,006	900	5,504	460
Morphological composition (% of forage DM)				
Leaves	53.3	7.77	33.4	11.6
Stem	36.3	5.95	45.2	3.67
Senescent material	10.3	1.48	21.4	7.91
Leaf:stem ratio	1.47	0.74	0.74	0.24

Table 4. Nutrient intake and digestibility for cows fed treatment diets (n = 22).

Item	Treatments[1]		SEM	*P*-value[2]
	CON	CSPF		Trt
Intake, kg/d				
DM	17.0	15.8	0.41	0.09
OM	15.3	14.2	0.35	0.05
NDF	6.44	5.72	0.23	0.04
CP	2.72	2.50	0.07	0.10
Total FA	0.61	0.79	0.03	<0.01
Digestibility, %				
DM	67.0	69.1	0.75	0.08
OM	71.8	75.2	0.94	0.04
NDF	53.0	57.6	1.79	0.03
CP	68.0	70.1	0.88	0.24
Total FA	76.4	80.1	1.00	0.02

[1] Treatments were: 1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[2] *P*-values associated with the effects of treatment.

Table 5. Energy intake, output and partitioning of cows fed treatment diets (n = 22).

	Treatments[1]		SEM	*P*-value[2]
Item	CON	CSPF		Trt
Energy intake[3], Mcal/d				
DE	52.4	51.2	1.05	0.43
ME	45.3	44.6	0.90	0.60
NE_L	28.6	28.4	0.57	0.75
Diet NE_L	1.69	1.79	0.02	0.01
Energy output, Mcal/d				
Milk[4]	16.2	18.0	0.69	0.07
Body reserves[5]	0.76	0.48	0.10	0.01
Grazing activities[6]	0.63	0.68	0.03	0.21
Maintenance[7]	10.8	8.97	0.65	0.04
Partitioning, % NE_L intake[8]				
Milk	56.6	64.4	2.27	0.01
Body reserves	2.70	1.79	0.11	<0.01
Grazing activities	2.26	2.40	0.08	0.44
Maintenance	36.8	28.7	2.31	<0.01

[1] Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[2] *P*-values associated with the effects of treatment.
[3] Diet energy values were calculated based on nutrient digestibility (Boerman et al., 2015a) using equations (NRC, 2001) according to Harvatine and Allen (2006) and multiplied by DMI to estimated energy intake.
[4] From NRC (2001): Milk energy output (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)].
[5] From NRC (2001): Body reserves output (Mcal/d) = [(2.88 + 1.036 × BCS) × ΔBW], where BCS was the average BCS for study and ΔBW was BW change.
[6] From NRC (2001): Grazing activities output (Mcal/d) = (Δd × 0.0004 × BW) + (0.0012 × BW), where Δd was the average distance (km) between pasture and milking center.
[7] From NRC (2001): Energy output for maintenance (Mcal/d) = NE_L intake (Mcal/d) − Milk energy output (Mcal/d) − Body reserves output (Mcal/d) – Grazing activities output (Mcal/d).
[8] Energy partitioning was calculated as the percentage of NE_L intake allocated to milk, body reserves, grazing activities, and maintenance (Boerman et al., 2015a).

Table 6. Plasma insulin and metabolites for cows fed treatment diets (n=22).

Item[1]	Treatments[2]		SEM	*P*-value[3]
	CON	CSPF		Trt
Insulin, µg/L	1.38	1.22	0.04	<0.01
NEFA, mmol/L	0.09	0.15	0.01	0.03
BHB, mmol/L	0.52	0.54	0.02	0.64
GGT, U/L	31.3	25.2	1.46	<0.01

[1]Gamma glutamyl transferase.
[2]Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[3] *P*-values associated with the effects of treatment.

Table 7. Milk yield, milk composition, BW, and BCS of cows fed treatment diets (n = 22).

Item	Treatments[1]		SEM	*P*-value[2]		
	CON	CSPF		Trt	Time	Trt×Time
Yield, kg/d						
Milk	21.1	22.7	0.94	0.03	<0.01	<0.01
Fat	0.71	0.78	0.03	0.04	<0.01	0.89
Protein	0.69	0.71	0.02	0.30	<0.01	0.12
Lactose	1.00	1.04	0.03	0.25	<0.01	0.14
3.5% FCM[3]	20.6	22.6	0.90	0.05	<0.01	0.45
ECM[4]	20.9	22.7	0.85	0.04	<0.01	0.24
Content, g/100g						
Fat	3.44	3.53	0.13	0.62	0.85	0.58
Protein	3.23	3.15	0.06	0.37	<0.01	0.26
Lactose	4.68	4.57	0.05	0.14	0.01	0.94
MUN, mg/dL	13.8	13.5	0.36	0.47	<0.01	0.45
BW, kg	495	509	12.6	0.28	<0.01	0.77
BW change kg/d	0.14	0.10	0.01	0.08	-	-
BCS	3.18	3.37	0.09	0.13	<0.01	0.16
BCS change	0.11	0.11	0.01	0.84	-	-

[1] Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[2] *P*-values associated with the effects of treatment, time, and treatment × time.
[3] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].

Table 8. Fatty acid concentration and yield by source of milk FA for cows fed treatment diets (n = 22).

Item	Treatments[1]		SEM	*P*-value[2]
	CON	CSPF		Trt
Summation by source[3], g/d				
De Novo	141	109	7.06	<0.01
Mixed	219	239	11.8	0.06
Preformed	281	327	18.0	0.03
Summation by source[3], g/100g				
De Novo	20.7	16.5	0.31	<0.01
Mixed	32.3	33.7	0.55	0.04
Preformed	41.2	45.0	0.77	<0.01

[1] Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[2] *P*-values associated with the effects of treatment.
[3] 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). Concentrations and yields of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

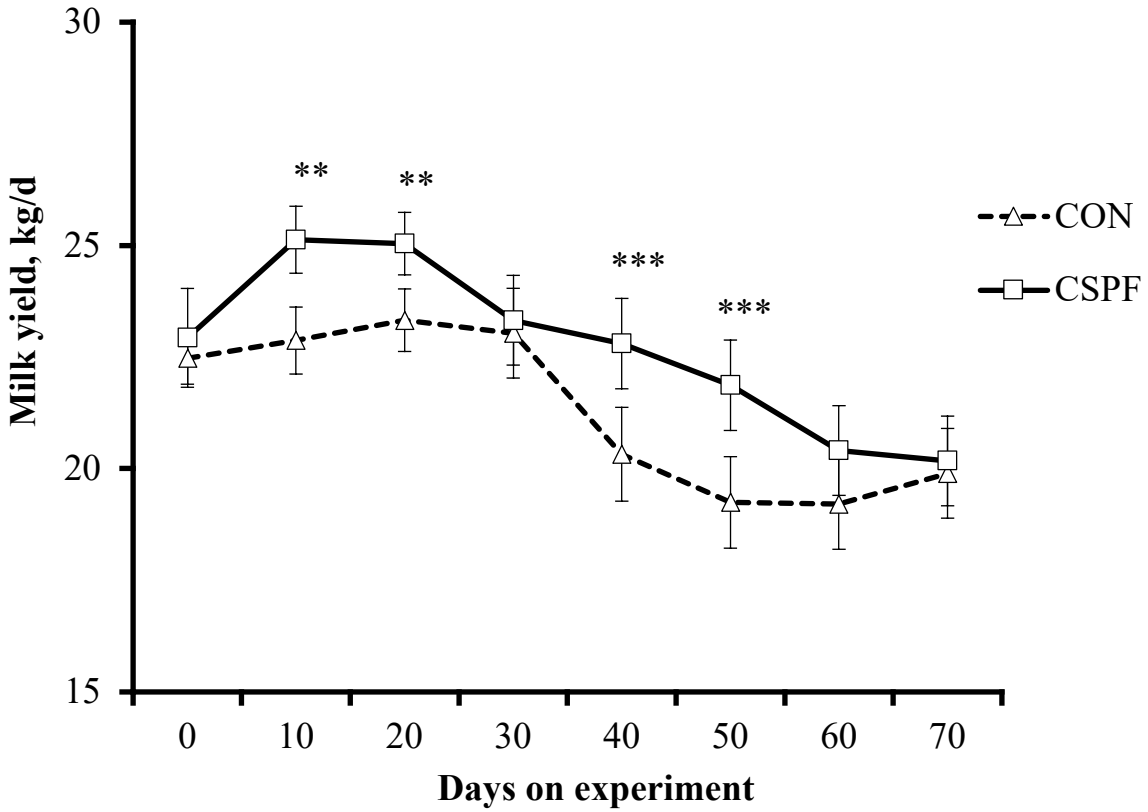

Figure 1. Milk yield over time. Treatments were: 1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement). Tendency at *$P \leq 0.10$ for each treatment effect. Significances at ** $P \leq 0.05$ and *** $P < 0.01$ for treatment effect. Error bars represent SEM.

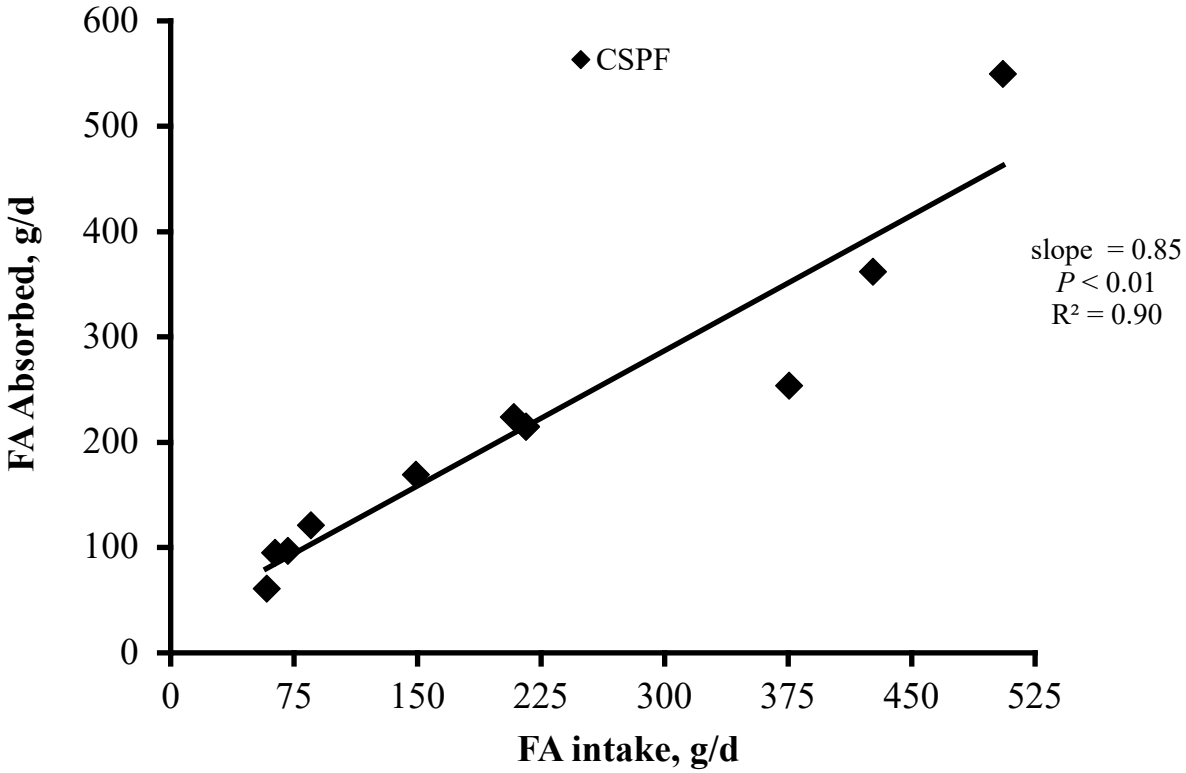

Figure 2. Lucas test to estimate total fatty acid (FA) digestibility of CSPF treatment. Linear and quadratic effects were tested. CSPF linearly increased ($P < 0.01$) FA absorption as supplemental FA intake increased. The slope represents the estimated digestibility of CSPF treatment, and the intercepts were not different from zero ($P = 0.25$).

Supplementary Table 1. Milk fatty acid yield for cows fed treatment diets (n=22).

	Treatments[1]			*P*-value[2]
Variable	CON	CSPF	SEM	Trt
Selected Individual FA, g/d				
C4:0	10.0	10.5	0.83	0.62
C6:0	10.0	7.97	0.52	<0.01
C8:0	6.44	4.55	0.31	<0.01
C10:0	15.2	10.5	0.78	<0.01
C11:0	0.46	0.35	0.02	<0.01
C12:0	18.7	14.5	0.91	<0.01
C14:0	72.9	57.5	3.82	<0.01
C14:1	7.03	4.46	0.51	<0.01
C15:0	7.27	5.52	0.24	<0.01
C16:0	209	229	11.4	0.05
cis-9 C16:1	10.9	10.3	0.59	0.81
C17:0	3.37	2.36	0.15	<0.01
C18:0	78.7	78.8	5.98	0.98
total *trans*-C18:1	15.1	19.8	0.93	<0.01
cis-9 C18:1	171	204	10.5	0.02
cis-9, *cis*-12 C18:2	11.5	15.7	0.80	<0.01
cis-9, *trans*-11 C18:2	4.73	7.04	0.46	<0.01
Summation by saturation, g/d				
SFA	432	410	22.3	0.36
MUFA	203	240	12.5	0.03
PUFA	16.2	21.9	1.11	<0.01
Summation of odd chain[3], g/d				
Linear	10.9	8.16	0.49	<0.01

[1] Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).
[2] *P*-values associated with the effects of treatment.
[3] Summation of odd fatty acid: C11:0, C15:0, and C17:0. These fatty acids were not used to estimate the summation by source. Yields and concentrations of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

Supplementary Table 2. Milk fatty acid content for cows fed treatment diets (n=22).

	Treatments[1]			*P*-value[2]
Item	CON	CSPF	SEM	Trt
Selected Individual FA, g/100g				
C4:0	1.50	1.45	0.04	0.35
C6:0	1.46	1.19	0.03	<0.01
C8:0	0.94	0.68	0.02	<0.01
C10:0	2.23	1.54	0.05	<0.01
C11:0	0.07	0.05	<0.01	<0.01
C12:0	2.77	2.19	0.06	<0.01
C14:0	10.6	8.46	0.23	<0.01
C14:1	1.01	0.65	0.05	<0.01
C15:0	1.03	0.79	0.02	<0.01
C16:0	30.8	32.4	0.54	0.03
cis-9 C16:1	1.48	1.38	0.05	0.16
C17:0	0.49	0.36	0.01	<0.01
C18:0	11.4	10.8	0.35	0.21
total *trans*-C18:1	2.26	2.88	0.08	<0.01
cis-9 C18:1	25.2	28.2	0.51	<0.01
cis-9, *cis*-12 C18:2	1.74	2.17	0.09	<0.01
cis-9, *trans*-11 C18:2	0.72	0.96	0.04	<0.01
Summation by saturation, g/100g				
SFA	63.6	59.4	0.65	<0.01
MUFA	29.8	33.1	0.56	<0.01
PUFA	2.43	3.12	0.12	<0.01
Summation of odd chain[3], g/100g				
Linear	1.59	1.20	0.02	<0.01

[1] Treatments were:1) CON (concentrate with no supplemental fat) 2) CSPF (concentrate with calcium salts of palm fatty acids supplement).

[2] *P*-values associated with the effects of treatment.

[3] Summation of odd fatty acid: C11:0, C15:0, and C17:0. These fatty acids were not used to estimate the summation by source. Yields and concentrations of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

4. INCREASING LEVELS OF CALCIUM SALTS OF PALM FATTY ACIDS AFFECT NUTRIENT DIGESTIBILITY, ENERGY PARTITIONING AND PRODUCTION RESPONSES OF EARLY-LACTATION GRAZING DAIRY COWS

INTERPRETATIVE SUMMARY

Increasing levels of calcium salts of palm fatty acids affect nutrient digestibility, energy partitioning and production responses of early-lactation grazing dairy cows. Our objective was to determine the dose response effects of calcium salts of palm fatty acids (**CSPF**) on nutrient digestibility, energy partitioning and production responses of early-lactation dairy cows grazing on tropical pastures, with a potentially positive carryover effect. Increasing CSPF to early-lactation dairy cows grazing on tropical pastures linearly increased NDF digestibility, quadratically increased fatty acid (**FA**) digestibility, and linearly increased energy output for milk, increasing the yields of milk, milk fat, and 3.5% FCM. Feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production.

ABSTRACT

The objective of our study was to determine the dose response effects of calcium salts of palm FA (**CSPF**) on nutrient digestibility, energy partitioning and production responses of early-lactation dairy cows grazing on tropical pastures. The carryover effect of CSPF supplementation was also evaluated during mid and late lactation. During the treatment period, all cows were kept in a grazing system. The treatments were offered for 90 d (treatment period), and consisted in the inclusion of four levels of CSPF: (1) **0** (control, 10 kg/d of concentrate without supplemental fat as fed-basis); (2) **0.2** (9.8 kg/d of control + 0.2 kg/d of CSPF as fed-basis), (3) **0.4** (9.6 kg/d of control + 0.4 kg/d of CSPF as fed-basis), (4) **0.6** (9.4 kg/d of control + 0.6 kg/d of CSPF as fed-basis). From 91 to 259 d all cows received a common diet without fat inclusion fed as TMR and the carryover effect was evaluated. Increasing CSPF linearly decreased DMI, and linearly increased NDF digestibility and quadratically increased fatty acid (**FA**) digestibility. CSPF linearly decreased plasma insulin concentration, decreased energy partitioning toward body reserves, and linearly increased energy output for milk, and the yields of milk, milk fat, milk lactose, and 3.5% FCM. CSPF linearly decreased de novo milk FA, and linearly increased mixed and preformed milk FA. We observed an interaction between CSPF and time for milk yield during carryover period. Compared with 0g/d, 0.6 kg/d of added CSPF promoted high milk production up until wk 29, and 0.4 kg/d of added CSPF promoted high milk production up until wk 25 after the end of the supplementation period. Increasing CSPF to early-lactation dairy cows grazing on tropical pasture increased NDF and FA digestibility, and linearly increased energy output for milk, increasing the yields of milk, milk fat, milk lactose, and 3.5% FCM. The increase in milk fat yield was driven by the increase in the yields of mixed and preformed milk FA. Feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production.

Keywords: Carryover effect, Fat supplements, Grazing systems, Tropical pastures

4.1. Introduction

During early lactation, the energy intake obtained by dairy cows cannot meet their requirements to support milk production (NRC, 2001). Compared with cows receiving a TMR diet, the detrimental effects of this negative energy balance period can be greater on grazing dairy cows, mainly on tropical pastures (Muller and Fales, 1998; Batistel et al., 2017; 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 feed as a means to combat this negative energy balance (da Silva et al., 2013; Batistel et al., 2017; de Souza et al., 2017).

Corn grain is the main concentrate supplement used for grazing dairy cows (Bargo et al., 2003), but feeding a supplemental fat as well has some advantages such as the reduced risk of acidosis and higher energy density (Schroeder et al., 2004). Overall, DMI of grazing cows is similar or decreases with added fat, while at the same time milk and FCM increase, suggesting an improvement in energy efficiency (Schroeder et al., 2004). However, research on grazing cows fed supplemental fat is scarce, and 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; de Souza et al., 2017).

Calcium salts of palm FA (**CSPF**) are one of the most common rumen inert fats used in dairy cows, which is mainly composed of palmitic (C16:0; ~45%) and oleic (*cis*-9 C18:1; ~35%) acids (Loften and Cornelius, 2004; de Souza et al., 2019). Oyadabe et al. (2019) reported that nutrient digestibility and milk yield of lactating dairy cows increased with the addition of CSPF compared with a prilled saturated FA in the form of triglyceride supplement. 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 was an effective strategy to alter energy partitioning, promoting increases in milk yield by an average of 4.25 kg/d from 3 to 16 wk postpartum (Batistel et al., 2017; de Souza et al., 2017), with a positive carryover effect up until 30 wk postpartum. Despite these positive effects, to our knowledge, no studies have been designed to evaluate the effects of a dose response of CSPF in early-lactation dairy cows grazing on tropical pastures.

Therefore, the objective of our study was to determine the dose response effects of CSPF on nutrient digestibility, energy partitioning and production responses of early-lactation dairy cows grazing on tropical pastures. The carryover effect of CSPF supplementation was also evaluated during mid and late lactation. Our hypothesis was that supplementing CSPF would increase NDF and FA digestibility, energy partitioning to milk, and the yield of milk and milk fat in early-lactation dairy cows, with a potentially positive carryover effect.

4.2. Materials and methods

4.2.1. Animal care

This study was conducted in Piracicaba, Sao Paulo, Brazil (22.7°S, 47.6°E and 546 m altitude) at the experimental farm of the University of Sao 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).

4.2.2. Design and Treatments

All animals received a common diet with no fat supplementation during a 15-d preliminary period to obtain baseline values. Forty multiparous dairy cows (Jersey × Holstein), with (mean ± SEM) 489 ± 14.5 kg of BW and 20 ± 5.0 DIM, were used in a randomized complete block design, and were blocked by milk yield, BCS and parity. During

the treatment period, all cows were kept in a grazing system. The treatments were offered for 90 d (**treatment period**), and consisted in the inclusion of four levels of calcium salts of palm fatty acids (**CSPF**; Nutri Gordura Lac, Nutricorp Inc., Araras, Sao Paulo, Brazil): (1) **0** (control, 10 kg/d of concentrate without supplemental fat as fed-basis); (2) **0.2** (9.8 kg/d of control + 0.2 kg/d of CSPF as fed-basis), (3) **0.4** (9.6 kg/d of control + 0.4 kg/d of CSPF as fed-basis), (4) **0.6** (9.4 kg/d of control + 0.6 kg/d of CSPF as fed-basis). Treatments were fed individually, using a tie-stall facility in 2 equal feedings at 0330 and 1530 h before milking. The fatty acids composition (**FA**) of treatments and pasture are shown in Table 1. The ingredients and nutrient composition of the pasture and concentrate supplements are shown in Table 2.

From 91 to 259 d all cows received a common diet without fat inclusion fed as TMR once a day at 0900 h (Table 2) (**carryover period**).

4.2.3. Grazing Management During Treatment Period

All cows grazed elephant grass (*Pennisetum purpureum* L. Cameroon) pastures as one herd, in an area of 5.0 ha, divided in paddocks with 0.2 ha with free access to natural shade and fresh water. Elephant grass was managed in a rotational system based on a canopy height, with 100 cm being the entry height target. At a canopy height of 100 cm, this elephant grass cultivar has 95% 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). Post-grazing target corresponded to approximately 50% of the entry height target. The paddocks were fertilized with 60 kg of N/ha after each grazing. 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 post-grazing height of the paddock was not reached, a group of dry cows was used to graze down the pasture to the target post-grazing height (50 cm).

4.2.4. Data and Sample Collection During Treatment Period

Pre- and post-grazing heights were measured every day at 20 randomized points before animals entered and after they left the paddock respectively (Table 3). Pre- and post-grazing forage mass were measured in 2 paddocks every 7 d by clipping the forage 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. Total forage 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 dried in a forced-air oven at 55°C for 72 h to determine the morphological composition of the forage mass (Table 3).

Forage and concentrate ingredients were collected for determination of their chemical composition every 7 d. Hand-plucked forage samples were taken at 20 randomized points before cows entered the paddock, by simulating the cows' grazing 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, Scientific, Philadelphia, PA).

Nutrient intake and digestibility were measured twice during study through total fecal estimation from titanium dioxide (**TiO2**), and the estimation of indigestible NDF (**iNDF**) content of feces and feeds. For 15 d in each intake estimation period (period 1: from 45 to 59 d, and period 2: from 75 to 89 d of experiment), cows received a daily dose of TiO2 (20 g/ cow per day), where half of total dose was received before each milking. In the last 5 d (period 1: 55 – 59 d, and period 2: 85 – 89 d), fecal grab samples were collected after morning and afternoon milkings, and immediately stored at −20°C. Samples were

subsequently thawed, dried at 55°C in a forced air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by cow.

Cows were milked twice a day at 0400 and 1600 h. Milk yield was recorded daily. Milk samples from both milkings were collected every 7 d and preserved with a bronopol preservative pill (Advanced Instruments, Norwood, MA). Milk samples used to determine milk FA profile were collected without preservative on 45 d and 90 d of experiment, and stored at -20°C. Cows were weighed every 7 d after morning milking and scored for body condition 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).

Blood samples were collected in the last day of the experiment (90 d) by venipuncture of coccygeal vessels with a sterile needle and vacuum tubes (~15 mL), immediately before the cows have received the morning treatment (0330 h). The samples were stored on ice until centrifugation at 2,000 × *g* for 15 min at 4°C. Subsequently, plasma was transferred into microcentrifuge tubes and stored at −20°C.

4.2.5. Sample Analysis and Calculations During Treatment Period

Forage, concentrate, and fecal samples were analyzed for DM by drying samples in an oven at 105°C for 24 h, ash (AOAC International, 2005; method 942.05), total N content by the Dumas combustion method using N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), NDF with sodium sulfite and heat-stable α-amylase (Van Soest et al., 1991), ADF and lignin (AOAC International, 2005; method 973.18), and fatty acids (**FA**, Sukhija and Palmquist, 1988).

Forage intake was estimated from total fecal excretion and feed indigestibility. To estimate fecal excretion, fecal samples were analyzed for titanium concentration according to Myers et al. (2004). The iNDF contents of forage, concentrate and fecal samples (NDF

remaining after 240 h of in situ incubation; Huhtanen et al., 1994) were determined to calculate indigestibility.

Milk samples were analyzed for fat, protein, lactose, and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Milk component yields were calculated from milk component contents for each milking and summed for a daily total. Yields of 3.5% fat-corrected milk (FCM) and energy-corrected milk (ECM) were calculated using the yields of milk and milk components as follow: 3.5% FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)]; and ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (NRC, 2001).

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 GC (GC Shimatzu 2010 with automatic injection, Shimadzu Corporation), equipped with a SP-2560 capillary column (100 m × 0.25 mm i.d. with 0.02 μm film thickness, Supelco, Bellefonte, PA) as previously described by Marques et al. (2019).

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 [Σ < C16], mixed [Σ C16 + C16:1], and preformed [Σ >C16]), saturation (Σ SFA, Σ MUFA, and Σ PUFA) and odd linear chain (Σ odd linear FA). Odd-branched-chain FA were not used in the summation by source.

Mean daily BW change (kg/d) and BCS change were calculated for each cow during the experimental period by linear regression (Boerman et al., 2015a). Energy concentration of the diet was calculated based on nutrient digestibility (Boerman et al., 2015a) using equations (NRC, 2001) according to Harvatine and Allen (2006a). Energy intake was calculated for each treatment from diet energy (DE, ME and NE_L) × DMI. Energy output (Mcal/d) for milk,

body reserves, and grazing activities were calculated according to NRC (2001) as: 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]; and Grazing activities output (Mcal/d) = (Δd × 0.0004 × BW) + (0.0012 × BW), where Δd was the average distance of 0.4 km between pasture and milking center, and ΔBW was BW change. Energy output for maintenance was calculated by difference as follow: Energy output for maintenance (Mcal/d) = NE_L intake (Mcal/d) − Milk energy output (Mcal/d) − Body reserves output (Mcal/d) – Grazing activities output (Mcal/d). Energy partitioning was calculated as the percentage of NE_L intake allocated to milk, body reserves, grazing activities, and maintenance (Boerman et al., 2015a). To estimate energy output and partitioning for each period of the intake estimation, milk composition, BW, BW change and BCS were estimated as described for each cow twice during study (period 1: from 28 to 59 d, and period 2: from 59 to 90 d of experiment).

Plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), ß-hydroxybutyrate (BHB), cholesterol, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), total protein, BUN, albumin, creatinine, triiodothyronine (T3), thyroxine (T4), and growth hormone (GH) were determined using a Multiskam MS ELISA reader (Labsystems, Helsinki, Finland), using enzyme-linked immunoassay (ELISA) methods.

4.2.6. Experimental Measures and Sample Collection During the Carryover Period

During carryover period, cows were milked twice a day at 0700 and 1700 h, and milk yield was recorded daily. Milk samples from both milkings were collected once a week and preserved with a bronopol preservative pill (Advanced Instruments, Norwood, MA). Feed ingredients were collected weekly and stored at −20°C. Subsequently, samples were thawed,

dried at 55°C in a forced air oven, ground through a 1-mm screen (Wiley mill, Thomas Scientific), and composited by month.

4.2.7. Statistical analysis (Treatment Period and Carryover Period)

Data were analyzed separately for treatment period (until 90 d) and carryover period (from 91 to 259 d). All data were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Production responses were analyzed with repeated measures according to the following model:

$$Yijkl = \mu + Fi + Tj + Bk + Cl\ (Bk) + Fi \times Tj + eijkl,$$

where Yijkl = the dependent variable, μ = the overall mean, Fi = the fixed effect of treatment, Tj = the fixed effect of time, Bk = the random effect of block, Cl (Bk) = the random effect of cow nested in block, Fi × Tj = the fixed effect of interaction between treatment and time, and eijkl = the residual error. The covariance structure used was selected based on the lowest Akaike's information criterion. Variables determined once (plasma hormones and metabolites) were analyzed without repeated measures, and without time and its interactions in the model. Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Orthogonal contrasts were used to test the linear, and quadratic effects of the treatments. Significant differences were declared at $P \leq 0.05$, and tendencies at $P \leq 0.10$ for main effects and interactions.

4.3. RESULTS

4.3.1. Nutrient Intake and Total-tract Digestibility During Treatment Period

We did not observe interactions between CSPF and time for any variable ($P > 0.05$, Table 4). Overall, CSPF affected the intakes of DM ($P = 0.05$), OM ($P = 0.04$), NDF ($P = 0.03$), CP ($P = 0.01$) and FA ($P < 0.01$, Table 4). Increasing CSPF linearly decreased the

intakes of DMI ($P = 0.02$), OM ($P = 0.01$), NDF ($P = 0.02$), and CP ($P < 0.01$), and increased FA intake ($P < 0.01$, Table 4).

CSPF had no effect on the digestibilities of DM ($P = 0.42$), OM ($P = 0.57$) and CP ($P = 0.55$), while it tended to affect NDF digestibility ($P = 0.09$), and affected FA digestibility ($P = 0.01$). CSPF linearly increased NDF ($P = 0.01$), and quadratically increased FA digestibility ($P < 0.01$, Table 4), with a maximum value obtained at approximately 0.4 kg of added CSPF (Figure 1).

4.3.2. Energy Intake, Energy Output, and Energy Partitioning During Treatment Period

We did not observe interactions between CSPF and time for any variable ($P > 0.05$, Table 5). In addition, CSPF had no effect on energy intake ($P > 0.05$, Table 5), and affected diet NE_L ($P < 0.01$). Increasing CSPF linearly increased diet NE_L ($P < 0.01$, Table 5).

CSPF tended to affect energy output for milk ($P = 0.09$), affected energy output for body reserves ($P < 0.01$), and had no effect on energy output for grazing activities ($P = 0.37$) and maintenance ($P = 0.83$, Table 5). CSPF linearly increased energy output for milk ($P = 0.01$) and linearly decreased energy output for body reserves ($P < 0.01$, Table 5).

CSPF had no effect energy partitioning toward grazing activities ($P = 0.96$), and it did affect energy partitioning toward body reserves ($P < 0.01$, Table 5). CSPF linearly decreased energy partitioning toward body reserves ($P < 0.01$, Table 5). Although increasing CSPF linearly increased energy partitioning toward milk ($P = 0.02$), we did not observe a treatment difference for this variable ($P = 0.15$, Table 5).

4.3.3. Blood Hormones and Metabolites During Treatment Period

Plasma concentrations of hormones and metabolites are shown in Table 6. We observed that CSPF affected insulin ($P = 0.02$), T4 ($P = 0.01$), NEFA ($P = 0.03$), and BHB (P

= 0.04). It tended to affect T3 (P = 0.09), and cholesterol (P = 0.07), and had no effect on the other variables (P > 0.05, Table 6). CSPF linearly decreased insulin (P = 0.04), T3 (P = 0.02), and T4 (P = 0.04), while on the other hand, it linearly increased GH (P <0.01), NEFA (P = 0.01), BHB (P = 0.01), and cholesterol (P = 0.01, Table 6).

4.3.4. Production Responses During Treatment Period

We did not observe interactions between CSPF and time for any variable (P > 0.05, Table 7). CSPF affected the yields of milk (P = 0.05), 3.5% FCM (P = 0.05), and the content of milk protein (P < 0.01). It tended to affect the yields of milk fat (P = 0.09), milk lactose (P = 0.09), and ECM (P = 0.08), and had no effect on the yield of milk protein (P = 0.54), nor on MUN (P = 0.51, Table 7). Increasing CSPF linearly increased the yields of milk (P = 0.01), milk fat (P = 0.01), milk lactose (P = 0.02), 3.5% FCM (P = 0.01), and ECM (P = 0.01, Table 7), and decreased the content of milk protein (P < 0.01, Table 7).

Overall, CSPF had no effect on BW (P = 0.92). We observed a tendency for interaction between treatment and time (P = 0.09, Table 7), however, there were no differences or tendencies among the treatments within weeks (P > 0.10). CSPF had no effect on BCS (P = 0.96, Table 7), but tended to affect BCS change (P = 0.09). BCS change linearly decreased with increasing CSPF (P = 0.03, Table 7).

4.3.5. 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 any variable (P > 0.05, Table 8). CSPF affected the yields of de novo (P = 0.05), mixed (P = 0.02), preformed (P = 0.03, Table 8), odd linear (P < 0.01), MUFA (P = 0.02), and milk

PUFA ($P < 0.01$, Supplementary Table 1). Increasing CSPF linearly decreased de novo and odd linear milk FA ($P < 0.01$, Supplementary Table 1), and linearly increased mixed, preformed (P < 0.01, Table 8), MUFA and milk PUFA (P < 0.01, Supplementary Table 1).

CSPF affected the yields of milk C4:0 ($P = 0.04$), C10:0 ($P = 0.04$), C11:0 ($P < 001$), C12:0 ($P = 0.04$), C14:1 ($P = 0.05$), C15:0 ($P = 0.05$), C16:0 ($P = 0.02$), total *trans*-C18:1 ($P < 0.01$), *cis*-9 C18:1 ($P = 0.01$) and *cis*-9, *cis*-12 C18:2 ($P < 0.01$, Supplementary Table 1). It tended to affect the yields of milk C8:0 ($P = 0.07$), C14:0 ($P = 0.09$), and C17:0 ($P = 0.06$), and had no effect on the yields of milk C6:0 ($P = 0.92$), *cis*-9 C16:1 ($P = 0.98$), and C18:0 ($P = 0.93$).

Increasing CSPF linearly decreased milk FA from 8 to 15-carbons, as well as milk C17:0 ($P \leq 0.05$ Supplementary Table 1), and linearly increased the yields of milk C4:0, C16:0, total *trans* C18:1, cis-9 C18:1, and *cis*-9, *cis*-12 C18:2 ($P < 0.01$, Supplementary Table 1). The results for milk FA concentration followed a similar pattern to those described for milk FA yield (Supplementary Table 2).

4.3.6. Production Responses During Carryover Period

We observed an interaction between CSPF and time for milk yield during carryover period ($P < 0.01$, Table 9, Figure 2). Compared with 0 kg/d, a 0.6 kg/g of added CSPF promoted high milk production up until wk 31 ($P < 0.05$), with no difference in wk 25. Compared with 0 g/d, a 0.4 kg/g of added CSPF promoted high milk production up until wk 25, but a difference was observed in wk 27 ($P < 0.05$, Figure 2). The inclusion of 0.2 kg/d of CSPF did not have a constant carryover effect on milk yield ($P > 0.05$, Figure 2).

We did not observe interactions between CSPF and time for any other variable during carryover period ($P > 0.05$, Table 9). CSPF had no effect on the yields of milk protein ($P = 0.13$), and ECM ($P = 0.22$), and on the contents of all milk components ($P > 0.05$, Table 9). CSPF tended to affect the yield of milk fat ($P = 0.06$) and affected the yields of milk

lactose (P < 0.01) and 3.5% FCM (*P* < 0.01, Table 9). Overall, we observed a positive linear effect of CSPF on the yields of milk fat (*P* = 0.01), milk lactose (*P* < 0.01) and 3.5% FCM (*P* < 0.01, Table 9).

4.4. Discussion

There is limited information on the impact of fat supplementation in dairy cows grazing on tropical pastures. Previous studies have shown that CSPF greatly increased milk and milk fat yield of early-lactation grazing dairy cows (Batistel et al., 2017; de Souza et al., 2017), but the dose response of CSPF is not well established. Therefore, our aim in the current study was to evaluate the effects of increasing levels of CSPF on nutrient digestibility, energy partitioning and production responses in early-lactation dairy cows which were simultaneously grazed on tropical pastures and fed a high corn concentrate. The carryover effects of CSPF supplementation was also evaluated.

We observed that increasing CSPF from 0 to 0.6 kg/d decreased DMI by 7.7%. Similarly, Harvatine and Allen (2006b) reported that increasing unsaturated FA from CSPF in dairy cow diets linearly decreased DMI by 15.1%. Recently, a meta-regression by Weld and Armentano (2017) observed that CSPF decreased DMI by 0.40 kg for each percentage unit of FA in the diet (%DM). Likewise, previous meta-analyses performed to evaluate the production responses of lactating dairy cows to different fat supplements found that CSPF decreased DMI by 0.64 (Rabbie et al., 2012), and 0.97 kg/d (Onetti and Grummer, 2004) compared with a non-fat supplemented control diet. The main reason for why CSPF decrease DMI is likely associated with the secretagogue action of unsaturated FA on the release of cholecystokinin and glucagon-like peptide-1, which acts by inhibiting gastric emptying (Allen, 2000; Relling and Reynolds, 2007; Bradford et al., 2008). In addition, human-related studies have indicated that T3 directly stimulates feeding at the level of the hypothalamus

(Kong et al., 2004). Interestingly, we observed that CSPF linearly decreased T3. Further studies are needed to better understand the impact of FA supplementation on the hormonal responses of lactating dairy cows and its relation to feed intake.

Feeding supplemental fat can also increase plasma NEFA concentration, increasing hepatic uptake and oxidation of NEFA in lactating cows (Allen 2000). This is more pronounced in early-lactation dairy cows when feed intake is depressed (Emery et al., 1992; Palmquist, 1994). Indeed, we observed that increasing CSPF increased plasma NEFA concentration, associated with a positive linear effect on plasma BHB concentration, which could indicate that part of the fat mobilization was directed to the liver, as BHB is formed from the incomplete oxidation of NEFA in the hepatic cells (Loor et al., 2007). However, it is unlikely that the reduction in DMI was mediated by an increase of NEFA oxidation, given that plasma concentration of BHB in our study was significantly lower than the values associated with metabolic alterations in dairy cows (higher than 1.2 mmol/L*;* Iwersen et al., 2013). Also, CSPF had no effect on plasma GGT and AST, potentially indicating that hepatic activity was not altered among the treatments (Rodriguez-Jimenez, 2018). Previous studies with early-lactation grazing dairy cows did not find effects of CSPF on DMI (Batistel et al., 2017; de Souza et al., 2017). There are few studies that evaluated DMI in grazing cows fed fat supplements, mainly due to the difficulties in estimating individual animal intake. Further studies are needed to better understand the impact of CSPF on DMI under grazing conditions.

A common concern regarding CSPF is the decrease in NE_L intake. In a review by Loften and Cornellius (2004), it was pointed out that low inclusions of calcium salts of FA in the diets could depress DMI to a level that would reduce daily NE_L intake. Nonetheless, in their evaluation the authors considered calcium salts of FA from both palm and soybean oil. Our results, however, indicated that the NE_L intake did not differ between treatments, because increasing CSPF from 0 to 0.6 kg/d linearly increased diet NE_L. On the other hand, de Souza

et al. (2017) reported that CSPF increased NE_L intake in early-lactation grazing dairy cows by 1.60 Mcal/d compared with a non-fat supplemented control treatment, and by 1.20 Mcal/d compared with a treatment with calcium salts of soybean oil.

Often, FA supplementation has decreased nutrient digestibility (Devendra and Lewis, 1974; Jenkins and Palmquist, 1984). Nonetheless, the negative effects are mainly associated with a very high fat inclusion, and a high load of unsaturated FA (Czerkawski, et al., 1966; Ikwuegbuet and Sutton, 1982; Rodrigues et al., 2019). Our results shown that CSPF had no effect on DM and OM digestibility, while at the same time it increased NDF digestibility. Inert fats have minimal impacts on rumen fermentation (Palmquist and Jenkins 2017). The most recent findings supplementing dairy cows with pure C16:0 supplements have demonstrated increments in NDF digestibility (de Souza et al., 2018; de Souza and Lock, 2018; de Souza and Lock, 2019). This could be related with the positive effect of C16:0 on fibrolytic bacteria, since this FA is an important component of the biomembrane cells of *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens* and *Ruminococcus albus* (Mackie 1991; Vlaeminck et al., 2006). Additionally, Piantoni et al. (2013) postulated that C16:0 supplementation increased NDF digestibility by decreasing passage rate. Both mechanisms can justify why CSPF linearly increased NDF digestibility in our study.

Increases in total FA duodenal flow usually decrease FA digestibility (Boerman et al., 2015b). We observed that CSPF had a positive linear effect on FA intake, and quadratically increased FA digestibility, with a maximum value obtained at approximately 0.4 kg/d of added CSPF. Inclusions of CSPF above that point started reducing FA digestibility. Our results support the hypothesis that up to a certain level, FA digestibility is more impacted 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). de Souza et al. (2018) reported that increasing *cis*-9 C18:1 increased total FA digestibility by increasing the digestibilities of 16-carbon and 18-

carbon FA compared with a saturated fat supplement (~40% C16:0 + ~40% C18:0). A meta-analysis using either ileal or fecal collection methods in lactating dairy cattle indicated that unsaturated FA have the highest apparent intestinal digestibility, with averages of 81.6, 77.7, and 79.2% for C18:1, C18:2 and C18:3 respectively, whereas the estimate for C18:0 was 73.0% (Boerman et al., 2015b). Therefore, the positive effects of CSPF on FA digestibility are due the unsaturated FA (*cis*-9 C18:1 and C18:2), which act as emulsifiers by increasing the micellar solubility, uptake and re-esterification of C18:0 in enterocytes (Freeman, 1969; Ockner et al., 1972, de Souza et al. 2019). However, our data indicated that this positive effect has a maximum value based on the amount of added fat. More research is needed to examine the interactions between the amount and profile of FA reaching the duodenum.

We observed that increasing CSPF linearly decreased plasma insulin concentration, which is possibly an effect of replacing dietary corn by CSPF. This effect was previously observed in other studies that replaced gluconeogenic with lipogenic ingredients in the diets of lactating dairy cows receiving TMR (Boerman et al., 2015a, van Knegsel et al. 2007). Insulin plays an important role in modulating energy partitioning of lactating dairy cows. High plasma insulin concentrations increase nutrient uptake by muscle and adipose tissue and decrease nutrient uptake by the mammary gland, which is not insulin-responsive (Bauman and Elliot, 1983). Furthermore, we observed that CSPF increased plasma NEFA concentration, and had a negative linear effect on energy output for body reserves, energy partitioning toward body reserves, BW change, and BCS change. Boerman et al. (2015a) also found that partially replacing starch with fiber and an enriched C16:0 supplement decreased insulin and energy partitioning toward body reserves. Although plasma insulin has not been evaluated, similar results for BW change and energy partitioning toward body reserves were observed by replacing starch with CSPF in early-lactation dairy cows grazing on tropical pastures (Batistel et al., 2017; de Souza et al., 2017). de Souza et al. (2017) reported that BW change decreased

by 8.7 kg when cows received CSPF from 3 to 16 wk of lactation compared with a decrease by 2.03 kg for cows that received a ground corn diet containing no supplemental fat. Batistel et al. (2017) observed that the BW change for cows fed CSPF increased 0.9 kg compared with an increase of 4.5 kg for cows fed a ground corn diet containing no supplemental fat from 3 to 16 wk of lactation. We observed an increase in BW change of 6.14 and 2.88 kg in cows receiving 0 or 0.6 kg/d of CSPF respectively.

Growth hormone also coordinates the nutrient partitioning of lactating dairy cows, but its physiological actions are different from insulin, resulting in increases in lipolysis, blood flow to the mammary gland, and nutrient uptake used for galactopoiesis (Davis, 1988; Bauman, 1992; Tucker, 2000). Interesting, we observed that CSPF linearly increased plasma GH concentration, energy output for milk, and the yields of milk, 3.5% FCM, ECM, and milk fat. In addition to these hormonal aspects, FA inclusion also 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). The reduction in de novo milk FA yield reduces 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). In this respect, we observed that CSPF had a positive linear effect on milk lactose yield. Lactose is the main osmotic regulator between the blood and alveolar lumen, and therefore the changes observed in milk yield are also expected to occur in milk lactose yield (Costa et al., 2019). Despite this and the effect of CSPF on insulin, we did not observe changes in plasma glucose concentration, which is expected due to the constant gluconeogenesis in the liver of ruminants (Aschenbach et al., 2010).

Moreover, 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). The thyroid hormones act by stimulating oxygen utilization and heat production in every cell of the body, resulting in an overall increase in the metabolic rate (Capen and Martin, 1989, Todini, 2007). In our study, we observed that increasing CSPF linearly reduced plasma concentrations of T3 and T4, indicating that partially replacing corn-based diets with FA may attenuate the heat stress typically observed in tropical grazing. Therefore, the energy spared with thermoregulatory functions can be used for milk production. Likewise, in a study with lactating dairy cows, Wang et al. (2010) observed that replacing fermentable carbohydrates with supplemental fat at 1.5% of diet DM decreased the body temperature caused by heat stress, saving a remarkable amount of metabolic heat, and increased the yields of milk and milk fat by 2.20 and 0.17 kg/d respectively.

Feeding CSPF did not affect the contents of milk fat and milk lactose but had a negative linear effect on the content of milk protein. These effects could have been the consequence of milk component dilutions, since CSPF linearly increased the yields of milk, milk fat, and milk lactose, but had no effect on the yield of milk protein. Similar results were observed in previous fat supplementation studies (Batistel et al., 2017; de Souza et al., 2017; de Souza et al., 2018).

Although CSPF increased the yield of milk fat, we observed a linear reduction in yield of de novo milk FA. de Souza et al. (2019) also observed that increasing dietary *cis*-9 C18:1 reduced the yield of de novo milk FA, and suggested that the increase of several *trans* FA in milk fat could indicate a mild milk fat depression (MFD) occurance. In our study, we did not detect levels of *trans*-10, *cis*-12 C18:2 in the milk fat of our samples, but it is important to consider that other FA produced as intermediates in rumen biohydrogenation have been shown to reduce milk fat (Bauman et al., 2011). It is also possible that CSPF reduced de novo milk FA yield because of the dietary increase in the exogenous long chain-FA (Palmquist, 2006; He and Armentano, 2011; He et al., 2012). Glasser et al. (2008) observed that the

inclusion of FA in the diets promotes a decrease in the novo milk FA and an increase in long-chain milk FA. Conversely, these authors suggested a positive relationship between de novo milk FA and long-chain milk FA in low fat diets.

Possibly, the mechanisms of FA substitution can be explained by the high specificity that the FA need to have when incorporated into the glycerol-3phosphate backbone (Jessen, 2002; Lindmark Månsson, 2008). Esterification of C16:0 is uniform between the positions sn-1 (44.1%) and sn-2 (45.2%) of the glycerol backbone, and more than 50% of de novo milk FA from 8 to 14-carbons are esterified at sn-2 (Jensen, 2002). Increasing CSPF linearly increased the yield of milk C16:0, which possibly resulted in the linear decrease in the yield of milk FA from 8 to 14 carbons, given that C16:0 competes with these FA to be incorporated at sn-2. Likewise, 41.5% of *cis*-9 C18:1, 98% of C4:0 and 93% of C6:0 are added at the sn-3 position of the glycerol backbone (Jensen, 2002). However, despite that we have observed that increasing CSPF linearly increased the yield of milk *cis*-9 C18:1, the yield of milk C4:0 increased, and the yield of milk C6:0 was not affected. The incorporation of de novo milk FA at sn-3 occurs by the enzyme diglyceride acyl transferase (DGAT), which is responsible for adding the final fatty acyl CoA at the sn-3 position to form the milk triglyceride. To begin this step, DGAT requires diacylglycerols as substrates, which are formed after the esterification of FA at sn-1 and sn-2 by the enzymes glycerol phosphate acyl transferase (GPAT) and acyl glycerol phosphate acyl transferase (AGPAT) respectively (Jessen, 2002; Glasser et l., 2008; Lock and de Souza 2018). As C16:0 is esterified at sn-1 and sn-2, possibly its linear increase into milk fat stimulated the uptake of C4:0 and C6:0 at sn-3, and thereby they were not decreased by the competition with *cis*-9 C18:1. This finding relies on the interdependence theory proposed by Glasser et al. (2008). However, in our study milk C4:0 and C6:0 would be driven by C16:0 in high fat diets, not in low fat diets as originally proposed. A possible explanation for this mechanism is that with the linear increase of C16:0 (melting point of 63.0

°C, Davis, 1990), the mammary gland may be prioritizing the synthesis or uptake of C4:0 and C6:0 to maintain milk fluidity at body temperature, regardless of the increase of *cis*-9 C18:1. Despite being an UFA, the melting point of C18:1 (13 °C) is greater than those of C4:0 (-5.3 °C) and C6:0 (-3.4 °C) (Davis, 1990; Scrimgeour and Harwood, 2007). Previous studies have also observed that feeding lactating dairy cows with an enriched C16:0 supplement increased milk C4:0 and had no effect on milk C6:0 (Piantoni et al., 2013; de Souza and Lock, 2018).

Increasing milk production of dairy cows during early lactation is a good strategy to increase farm profits. Roche et al. (2013) reported that increasing one extra kilogram of milk at the peak of lactation can result in more 200 kg of milk throughout the whole lactation period. Thus, we also evaluated the carryover effect of CSPF supplementation in early-lactation dairy cows grazing on tropical pastures. Our study demonstrated that 0.4 and 0.6 kg/d of added CSPF promoted a higher milk yield at the end of the supplementation period. However, the magnitude of the response was different between these doses. A dose of 0.6 kg/d had a positive carryover effect up until wk 31, and a dose of 0.4 kg/d had a positive carryover effect up until wk 25. 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 post-partum (Batistel et al., 2017, de Souza et al., 2017). One of the possible reasons for the carryover effect on milk yield is associated with the increment in nutrient availability to the mammary gland, which would stimulate secretory cells and increase cell proliferation in the mammary gland, promoting greater lactation persistency (Knight, 2000; Nørgaard et al., 2005). This hypothesis can also justify why carryover effects respond better to nutritional manipulations in early-lactation dairy cows, as long as this lactation stage promotes the largest energy partition toward mammary gland. Our results support that CSPF consistently increased nutrient availability to the mammary gland over a

long period of supplementation, which may have caused the positive carryover for 0.4 and 0.6 kg/d of added CSPF.

In addition, it is also important to consider the hormonal and metabolic changes that occurred during the CSPF supplementation. Several studies have reported that bST promotes a greater peak milk yield and an increased persistency in yield over the lactation cycle. Overall, when the treatment with bST is concluded, milk yield does not return immediately to the pretreatment levels, and a positive carryover effect can be observed (Peel and Bauman, 1987; Bauman and Vernon, 1993). This indicates that the responses obtained in plasma GH concentration in our study may have influenced the positive carryover effect on milk yield. As a homeorhetic controller (Bauman, 1992), it is possible that GH has a central role in increasing long-term nutrient availability to the mammary gland, resulting in the aforementioned effects on the mammary cells. In fact, it has been demonstrated that GH increases the activity per secretory cell and reduces the loss of secretory cells in the mammary gland (Bauman, 1992). Further studies are needed to understand the impact of CSPF supplementation on carryover period.

4.5. CONCLUSION

Increasing CSPF to early-lactation dairy cows grazing on tropical pastures had no effect on energy intake, linearly increased NDF and quadratically increased FA digestibility, linearly decreased plasma insulin concentration, linearly increased plasma GH concentration and energy output for milk, promoting linear increases in the yields of milk, milk fat, 3.5% FCM, and ECM. The increase in milk fat yield was driven by the increase in the yields of mixed and preformed milk FA. Also, feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production.

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Table 1. Fatty acid (FA) composition supplements of concentrate and forage fed during treatment period[1].

	Fat supplement[2]	Concentrate[3]	Forage[4]
FA, %	78.2	2.86	3.85
FA profile of each treatment, g/100g FA			
C12:0	3.83	0.20	0.87
C14:0	1.72	0.20	0.15
C16:0	44.0	24.5	21.4
C18:0	4.16	3.81	2.18
cis-9 C18:1	38.0	39.2	0.00
cis-9, *cis*-12 C18:2	7.56	34.7	10.6
cis-9, *cis*-12, *cis*-15 C18:3	0.24	0.47	53.3

[1] Average (n=13) composition of FA based on samples taken during the collection period.
[2] Calcium salts of palm fatty acids; Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil.
[3] Ground corn, soybean meal, mineral and vitamin premix (Nutrient composition in Table 2).
[4] Elephant grass (*Pennisetum purpureum* L. Cameroon; nutrient composition in Table 2).

Table 2. Ingredient and nutrient composition of forage and treatments.

Item	Forage[1]	CSPF				Carryover diet
		0	0.2	0.4	0.6	
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	4.68	2.00
Fat supplement[4]		0.00	2.17	4.33	6.49	
Corn silage						60.0
Urea						0.4
Nutrient Composition, % DM						
OM	87.5	91.9	91.5	91.1	90.8	94.2
NDF	59.4	14.9	14.6	14.3	13.9	37.6
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	19.9	13.2	12.9	12.7	12.4	16.9
Ash	11.7	8.00	8.38	8.76	9.14	5.70
FA	3.85	2.86	4.20	5.40	6.87	2.32
12:0	0.03	0.01	0.07	0.12	0.23	
14:0	0.01	0.01	0.04	0.06	0.11	
16:0	0.82	0.67	1.33	1.98	3.29	
18:0	0.08	0.10	0.16	0.22	0.34	
cis-9 18:1	0.00	1.07	1.63	2.19	3.31	
cis-9, *cis*-12 18:2	0.41	0.95	1.06	1.17	1.39	
cis-9, *cis*-12, *cis*-15 18:3	2.05	0.01	0.02	0.02	0.03	

[1]Elephant grass (*Pennisetum purpureum* L. Cameroon)
[2]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[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 D3, 1,250 of vitamin E.
[4]Calcium salts of palm fatty acids; Nutri Gordura Lac, Nutricorp Inc., Araras, São Paulo, Brazil.

Table 3. Pasture characteristics at pre- and post-grazing.

	Pre-grazing	SD	Post-grazing	SD
Sward height (cm)	98.2	2.35	56.9	2.90
Forage mass (kg of DM/ha)	8,089	9,12.0	4,551	485
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

.

Table 4. Nutrient intake and digestibility for cows fed treatment diets (n = 40).

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Intake, kg/d										
DMI	16.9	15.7	15.9	15.6	0.38	0.05	0.01	0.56	0.02	0.21
OM	15.2	14.1	14.3	14.0	0.33	0.04	0.01	0.56	0.01	0.20
NDF	6.00	5.26	5.34	5.14	0.22	0.03	0.02	0.56	0.01	0.21
CP	2.68	2.41	2.43	2.35	0.08	0.01	0.01	0.55	<0.01	0.21
Total FA	0.68	0.74	0.86	0.97	0.02	<0.01	0.01	0.56	<0.01	0.21
Digestibility, %										
DM	65.9	66.9	67.0	67.5	0.75	0.42	0.97	0.70	0.11	0.73
OM	71.1	72.0	72.3	72.5	1.15	0.57	0.25	0.57	0.18	0.68
NDF	53.0	55.6	56.6	58.2	1.65	0.09	0.04	0.95	0.01	0.71
CP	66.9	65.8	64.8	65.2	1.13	0.55	0.26	0.52	0.21	0.49
Total FA	74.2	76.9	79.7	77.2	1.08	0.01	0.05	0.94	0.01	<0.01

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[2]*P*-values associated with the effects of treatment, time, and treatment × time.
[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.

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

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item[4]	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Energy intake[5], Mcal/d										
DE	51.5	50.0	50.9	51.0	1.17	0.78	<0.01	0.35	0.88	0.45
ME	44.4	43.5	44.3	44.5	1.04	0.83	<0.01	0.34	0.80	0.53
NE_L	28.0	27.6	28.1	28.3	0.67	0.82	0.00	0.34	0.57	0.59
Diet NE_L	1.67	1.76	1.77	1.82	0.03	0.00	0.77	0.93	<0.01	0.38
Energy output, Mcal/d										
Milk[6]	15.8	16.2	17.7	18.6	1.68	0.09	0.03	0.79	0.01	0.73
Body reserves[7]	0.38	0.28	0.22	0.17	0.01	<0.01	0.75	0.27	<0.01	0.08
Grazing activities[8]	0.66	0.63	0.69	0.70	0.36	0.37	0.56	0.58	0.21	0.55
Maintenance[9]	11.0	10.1	9.94	9.74	2.04	0.83	0.42	0.76	0.40	0.75
Partitioning, % NE_L intake[10]										
Milk	54.4	60.4	62.0	65.9	6.86	0.15	0.17	0.43	0.02	0.74
Body reserves	1.33	1.03	0.79	0.59	0.03	<0.01	0.21	0.57	<0.01	0.07
Grazing activities	2.41	2.36	2.45	2.44	0.14	0.96	0.82	0.49	0.76	0.87
Maintenance	38.9	35.8	34.1	32.8	6.95	0.51	0.15	0.68	0.14	0.77

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[2]*P*-values associated with the effects of treatment, time, and treatment × time.
[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.

[4] To estimate energy output and partitioning in every period of intake estimation (period 1 and 2), we used the values of milk composition, BW, and BW change obtained in period 1 (from 28 to 59 d), and period 2 (from 59 to 90 d).
[5]Diet energy values were calculated based on nutrient digestibility (Boerman et al., 2015a) using equations (NRC, 2001) according to Harvatine and Allen (2006a) and multiplied by DMI to estimated energy intake.
[6]From NRC (2001): Milk energy output (Mcal/d) = [9.29 × fat (%) + 5.63 × true protein (%) + 3.95 × lactose (%)].
[7]From NRC (2001): Body reserves output (Mcal/d) = [(2.88 + 1.036 × BCS) × ΔBW], where BCS was the average BCS, and ΔBW was BW change.
[8]From NRC (2001): Grazing activities output (Mcal/d) = (Δd × 0.0004 × BW) + (0.0012 × BW), where Δd was the average distance (km) between pasture and milking center.
[9]From NRC (2001): Energy output for maintenance (Mcal/d) = NE_L intake (Mcal/d) − Milk energy output (Mcal/d) − Body reserves output (Mcal/d) – Grazing activities output (Mcal/d).
[10]Energy partitioning was calculated as the percentage of NE_L intake allocated to milk, body reserves, grazing activities, and maintenance (Boerman et al., 2015a).

Table 6. Plasma hormones and metabolites for cows fed treatment diets (n=40).

Item[1]	CSPF[2]				SEM	*P*-value[3]	Contrasts[4]	
	0	0.2	0.4	0.6		Trt	Linear	Quadratic
Insulin, µg/L	1.15	1.06	1.09	1.04	0.02	0.02	0.04	0.45
Glucose, mg/dL	56.6	59.8	57.2	57.9	1.94	0.61	0.88	0.50
Total protein, g/dL	9.27	9.00	9.03	8.78	0.21	0.43	0.13	0.95
Albumin, g/dL	3.24	3.32	3.22	3.21	0.10	0.84	0.66	0.67
BUN, mg/dL	33.7	34.1	32.2	33.8	1.76	0.88	0.82	0.76
Creatinine, mg/dL	1.19	1.08	1.39	1.38	0.13	0.24	0.13	0.71
Cholesterol, mg/dL	160	182	199	222	18.1	0.07	0.01	0.95
NEFA, mmol/L	0.12	0.14	0.15	0.16	0.01	0.03	0.01	0.47
BHB, mmol/L	0.42	0.50	0.51	0.61	0.05	0.04	0.01	0.77
AST, U/L	76.9	61.3	57.6	73.2	10.1	0.47	0.73	0.13
GGT, U/L	26.4	25.7	24.7	23.3	1.75	0.60	0.19	0.84
T3, ng/mL	1.20	1.08	0.95	0.93	0.09	0.09	0.02	0.54
T4, µg/dL	4.72	4.58	3.86	4.33	0.26	0.01	0.04	0.11
GH, µUi/mL	1.08	1.09	1.12	1.15	0.01	0.02	<0.01	0.56

[1] GGT = gamma glutamyl transferase, AST = aspartate aminotransferase, T3 = triiodothyronine, T4 = thyroxine, GH = growth hormone.
[2]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[3]*P*-values associated with the effects of treatment.
[4] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.

Table 7. Milk yield, milk composition, BW, and BCS of cows fed treatment diets (n = 40).

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Yield, kg/d										
Milk	22.9	23.5	24.2	26.3	1.63	0.05	<0.01	0.99	0.01	0.44
Fat	0.75	0.77	0.82	0.88	0.05	0.09	<0.01	0.99	0.01	0.63
Protein	0.73	0.70	0.73	0.75	0.04	0.54	<0.01	0.99	0.32	0.29
Lactose	1.06	1.09	1.11	1.22	0.07	0.09	<0.01	0.98	0.02	0.36
3.5% FCM[4]	22.0	22.8	23.8	25.9	1.43	0.05	<0.01	0.99	0.01	0.51
ECM[5]	22.5	22.8	23.8	25.6	1.38	0.08	<0.01	0.94	0.01	0.41
Content, g/100g										
Fat	3.33	3.34	3.36	3.25	0.1	0.87	0.06	0.65	0.61	0.55
Protein	3.20	3.05	3.00	2.82	0.07	<0.01	<0.01	0.27	<0.01	0.79
Lactose	4.66	4.64	4.6	4.56	0.06	0.66	<0.01	0.32	0.21	0.87
MUN, mg/dL	14.0	13.6	13.2	13.1	0.57	0.51	<0.01	0.99	0.14	0.76
BW, kg	500	490	495	494	14.6	0.92	<0.01	0.09	0.79	0.67
BW change kg	6.14	4.81	3.81	2.88	1.41	0.10	-	-	0.03	0.83
BCS	3.32	3.36	3.35	3.31	0.13	0.96	0.02	0.80	0.92	0.95
BCS change	0.05	0.02	0.02	0.01	0.09	0.09	-	-	0.03	0.25

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg/d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).

[2]*P*-values associated with the effects of treatment, time, and treatment × time.

[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.

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

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

Table 8. Fatty acid concentration and yield by source of milk FA for cows fed treatment diets (n = 40).

	CSPF[1]					*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6	SEM	Trt	Time	Trt×Time	Linear	Quadratic
Summation by source[4], g/d										
De Novo	167	153	141	131	10.7	0.05	0.32	0.27	<0.01	0.82
Mixed	234	255	269	299z	18.6	0.02	0.63	0.32	<0.01	0.76
Preformed	324	327	361	384	24.1	0.03	<0.01	0.73	<0.01	0.55
Summation by source[4], g/100g										
De Novo	21.6	19.8	17.4	16.2	0.60	<0.01	0.02	0.39	<0.01	0.56
Mixed	30.8	32.6	33.6	34.8	0.84	<0.01	<0.01	0.77	<0.01	0.73
Preformed	42.2	43.3	44.7	46.1	0.99	0.04	0.03	0.79	<0.01	0.94

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[2]*P*-values associated with the effects of treatment, time, and treatment × time.
[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.
[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). Concentrations and yields of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

Table 9. Milk yield and milk composition of cows during carryover period (n = 40).

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Yield, kg/d										
Milk	16.8	17.0	18.0	19.2	2.37	0.84	<0.01	<0.01	0.38	0.80
Fat	0.57	0.64	0.66	0.67	0.03	0.06	0.44	0.48	0.01	0.35
Protein	0.57	0.57	0.61	0.58	0.02	0.13	0.07	0.91	0.22	0.30
Lactose	0.75	0.77	0.84	0.85	0.01	<0.01	0.21	0.54	<0.01	0.95
3.5% FCM[4]	17.1	17.3	18.9	19.5	0.67	<0.01	0.45	0.47	<0.01	0.70
ECM[5]	17.8	17.7	18.6	18.9	0.53	0.22	0.21	0.93	0.05	0.76
Content, g/100g										
Fat	3.55	3.59	3.60	3.64	0.19	0.98	0.19	0.52	0.70	0.98
Protein	3.43	3.28	3.31	3.13	0.10	0.13	0.04	0.93	0.03	0.92
Lactose	4.49	4.51	4.46	4.44	0.07	0.90	0.21	0.60	0.51	0.84
MUN	15.4	15.2	14.1	14.2	0.81	0.45	0.11	0.93	0.14	0.84

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[2]*P*-values associated with the effects of treatment, time, and treatment × time.
[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.
[4]Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].
[5]Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)].

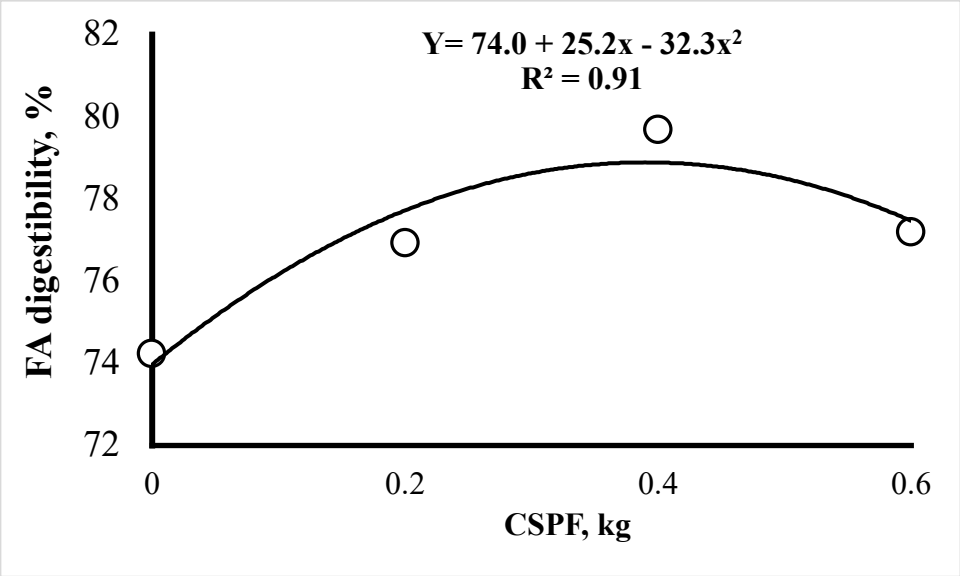

Figure 1. Total fatty acid digestibility. Calcium salts of palm fatty acid (CSPF) levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).

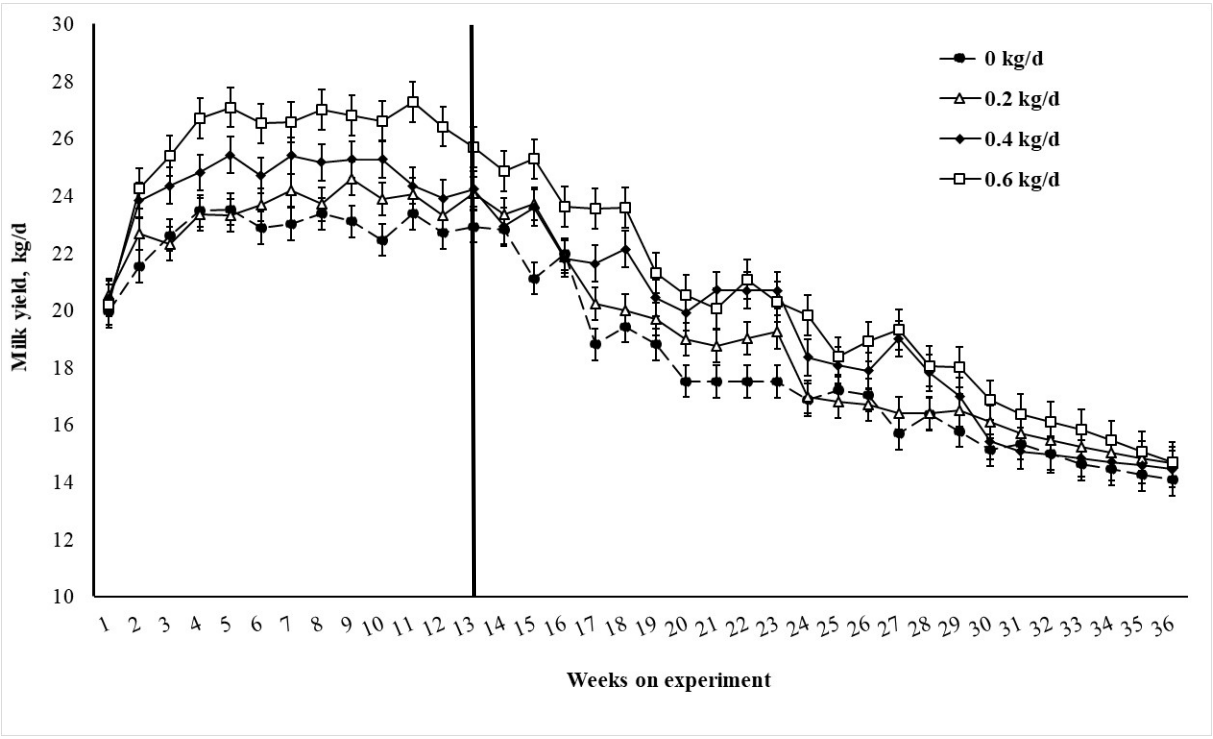

Figure 2. Calcium salts of palm fatty acid (CSPF) levels (0, 0.2, 0.4 and 0.6 kg/d) over time during treatment period (from 1 to 12 wk) and carryover periods (from 13 to 36 wk). Error bars represent SEM.

Supplementary Table 1. Milk fatty acid yield for cows fed treatment diets (n=40).

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Selected Individual FA, g/d										
C4:0	11.9	12.6	13.3	15.0	1.17	0.04	<0.01	0.12	<0.01	0.51
C6:0	11.8	11.7	11.1	11.8	1.04	0.92	0.06	0.65	0.83	0.65
C8:0	8.02	7.62	7.62	6.34	0.56	0.07	0.75	0.15	0.01	0.99
C10:0	19.6	18.8	15.2	15.3	1.44	0.04	0.03	0.46	<0.01	0.65
C11:0	0.87	0.73	0.68	0.44	0.15	<0.01	0.81	0.23	<0.01	0.13
C12:0	23.6	23.3	19.5	18.6	1.60	0.04	0.64	0.37	<0.01	0.90
C14:0	81.4	76.8	69.8	66.6	5.10	0.09	0.26	0.22	0.02	0.77
C14:1	6.32	5.23	4.86	4.59	0.50	0.05	<0.01	0.17	<0.01	0.37
C15:0	8.44	7.70	7.10	6.46	0.61	0.05	0.58	0.11	<0.01	0.94
C16:0	224	245	259	288	17.6	0.02	0.53	0.33	<0.01	0.73
cis-9 C16:1	10.1	10.1	10.5	10.3	1.00	0.98	0.15	0.44	0.82	0.90
C17:0	3.95	3.46	3.28	3.28	0.24	0.06	0.35	0.29	0.05	0.27
C18:0	96.7	102	102	103	9.16	0.93	<0.01	0.96	0.62	0.76
total *trans*-C18:1	18.9	20.7	22.7	26.8	1.72	<0.01	<0.01	0.83	<0.01	0.40
cis-9 C18:1	189	190	209	231	14.4	0.01	0.05	0.61	<0.01	0.26
cis-9, *cis*-12 C18:2	13.4	14.0	16.8	17.3	1.01	<0.01	<0.01	0.31	<0.01	0.91
Summation by saturation, g/d										
SFA	491	509	507	541	37	0.67	0.15	0.51	0.25	0.78
MUFA	225	227	248	274	16.8	0.02	<0.01	0.51	<0.01	0.27
PUFA	18.1	18.2	22.3	23.4	1.29	<0.01	0.02	0.25	<0.01	0.59
Summation of odd chain[4], g/d										
Linear	13.3	11.9	10.7	8.00	0.95	<0.01	0.28	0.59	<0.01	0.42

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).

[2]*P*-values associated with the effects of treatment, time, and treatment × time.

[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.

[4]Summation of odd fatty acid: C11:0, C15:0, and C17:0. These fatty acids were not used to estimate the summation by source. Yields and concentrations of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

Supplementary Table 2. Milk fatty acid content for cows fed treatment diets (n=40).

	CSPF[1]				SEM	*P*-value[2]			Contrasts[3]	
Item	0	0.2	0.4	0.6		Trt	Time	Trt×Time	Linear	Quadratic
Selected Individual FA, g/100g										
C4:0	1.54	1.61	1.63	1.65	0.06	0.30	<0.01	0.15	0.10	0.55
C6:0	1.55	1.49	1.37	1.33	0.05	0.01	0.34	0.56	<0.01	0.86
C8:0	1.05	0.98	0.83	0.78	0.04	<0.01	0.76	0.71	<0.01	0.87
C10:0	2.59	2.37	1.88	1.7	0.13	<0.01	0.41	0.44	<0.01	0.87
C11:0	0.11	0.10	0.08	0.05	0.02	<0.01	0.71	0.19	<0.01	0.22
C12:0	3.13	2.96	2.43	2.37	0.15	<0.01	0.10	0.30	<0.01	0.7
C14:0	10.7	9.76	8.65	8.10	0.25	<0.01	<0.01	0.42	<0.01	0.42
C14:1	0.84	0.68	0.6	0.56	0.06	<0.01	<0.01	0.26	<0.01	0.25
C15:0	1.12	0.98	0.89	0.75	0.05	<0.01	0.50	0.36	<0.01	0.99
C16:0	29.0	31.0	32.0	34.0	0.81	<0.01	0.01	0.73	<0.01	0.74
cis-9 C16:1	1.33	1.28	1.27	1.20	0.08	0.57	0.02	0.62	0.18	0.89
C17:0	0.52	0.44	0.41	0.36	0.02	<0.01	0.67	0.35	<0.01	0.35
C18:0	12.8	12.9	12.6	11.8	0.57	0.51	<0.01	0.34	0.20	0.38
total *trans*-C18:1	2.47	2.68	2.88	3.11	0.15	0.02	<0.01	0.97	<0.01	0.94
cis-9 C18:1	24.9	24.6	26.2	27.0	0.84	0.1	0.17	0.91	0.02	0.48
cis-9, *cis*-12 C18:2	1.76	1.85	2.12	2.01	0.09	0.02	0.06	0.70	0.01	0.24
Summation by saturation, g/100g										
SFA	64.5	65.0	62.9	62.5	0.98	0.21	0.45	0.87	0.07	0.64
MUFA	29.6	29.3	30.9	31.9	0.87	0.10	0.20	0.92	0.02	0.43
PUFA	2.37	2.42	2.82	2.73	0.13	0.02	0.35	0.64	<0.01	0.57
Summation of odd chain[4], g/100g										
Linear	1.76	1.52	1.33	0.93	0.08	<0.01	0.04	0.80	<0.01	0.35

[1]Treatments were four calcium salts of palm fatty acids levels: 1) 0 (control, 10 kg/d of concentrate without supplemental fat as fed basis); 2) 0.2 (9.8 kg/d of control + 0.2 kg /d of CSPF as fed basis), 3) 0.4 (9.6 kg/d of control + 0.4 kg/d of CSPF as fed basis), and 4) 0.6 (9.4 kg/d of control + 0.6 kg/d of CSPF as fed basis).
[2]*P*-values associated with the effects of treatment, time, and treatment × time.
[3] Contrasts evaluated refers to the linear, and quadratic effects of CSPF inclusion.
[4]Summation of odd fatty acid: C11:0, C15:0, and C17:0. These fatty acids were not used to estimate the summation by source. Yields and concentrations of individual fatty acids are reported in Supplementary Tables 1 and 2, respectively.

5. CONCLUSIONS

Results reported in this thesis have examined the effects of calcium salts of palm fatty acids (**CSPF**) on nutrient digestibility, energy partitioning and production responses of lactating dairy cows. In chapter 2 we performed 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 receiving TMR. In addition, change-over designs (Crossover and Latin square) have been excluded from previous meta-analyses on fatty acid (**FA**) supplementation, because of the concerns related with potential carryover effects. Therefore, we performed another meta-analysis to evaluate whether experimental designs impact the responses of supplemental CSPF. Feeding CSPF to lactating dairy cows receiving TMR reduced DMI, increased NDF digestibility, and increased the yields of milk, milk fat and 3.5% FCM. The increase in milk fat yield was driven by the increase in the yields of mixed and preformed milk FA. Also, we did not find any reason for the restrictive use of change-over designs in CSPF supplementation studies and meta-analyses.

Two other studies were performed to evaluate the effects of CSPF on nutrient digestibility, energy partitioning, and production responses of lactating dairy cows grazing on tropical pastures. In chapter 3, we evaluated the effects of CSPF in mid-lactation dairy cows. Feeding CSPF to mid-lactation dairy cows grazing on tropical pastures had no effect on energy intake, increased OM, NDF and FA digestibility, decreased plasma insulin concentration, and altered energy partitioning, promoting increases in the yields of milk, milk fat, 3.5% FCM, and ECM.

In chapter 4, we evaluated the dose response effects of CSPF in early-lactation dairy cows grazing on tropical pastures, with a potentially positive carryover effect. Increasing CSPF in early-lactation dairy cows had no effect on energy intake, linearly increased NDF and quadratically increased FA digestibility, linearly decreased plasma insulin concentration,

linearly increased plasma GH concentration and energy output for milk, promoting linear increases in the yields of milk, milk fat, 3.5% FCM, and ECM. We also observed that CSPF linearly decreased plasma concentrations of T3 and T4, potentially indicating that FA supplementation may attenuate the heat stress typically observed in tropical grazing. Feeding CSPF to early-lactation dairy cows grazing on tropical pastures had a positive carryover effect on milk production. We hypothesized that this carryover effect may be modulated by GH, which would act by increasing nutrient availability to the mammary gland over a long period of time, resulting in an increase in the mammary cell number and cell secretory activity. Nonetheless, the mechanisms related with carryover effect due to FA supplementation deserve future investigation.

Altogether, these studies increased our understanding of CSPF supplementation on nutrient digestibility, energy partitioning and production responses of lactating dairy cows. We also contributed to understand whether experimental designs impact the responses of supplemental CSPF by demonstrating that change-over designs can be used in CSPF supplementation studies and meta-analyses. Our results indicate that CSPF supplementation is a good strategy to increase the yields of milk and milk components in both lactating dairy cows receiving TMR and grazing dairy cows. The positive responses to CSPF supplementation occurred in both mid and early-lactation dairy cows grazing on tropical pastures. The CSPF supplementation in early-lactation dairy cows grazing on tropical pastures can be even more advantageous due the positive carryover effect. Our findings have potential to positively impact the dairy industry, as they are useful to improve nutritional management practices, resulting in increased farm profits.