University of São Paulo
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Performance and metabolism of early lactation dairy cows receiving doses of calcium salts of palm oil supplemented or not with Lysolecithin

Djonatan Machado

Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

Piracicaba
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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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CONTENTS

RESUMO

Desempenho e metabolismo de vacas leiteiras em início de lactação recebendo doses de sais de cálcio de ácidos graxos do óleo de palma associadas ou não com a lisolecitina

Os objetivos do presente estudo foram avaliar os efeitos da suplementação com doses de sais de cálcio de ácido graxo do óleo de palma com ou sem Lisolecitina sobre as respostas produtivas, digestibilidade dos nutrientes, ingestão e partição energética, respostas metabólicas e respostas ruminais de vacas em início de lactação em confinamento. Quarenta e quatro vacas Holandês ou ¾ Holandês x ¼ Jersey, sendo 16 primíparas e 28 multíparas, em início de lactação (média ± DP no início do experimento; DEL = 20 ± 4 e 20 ± 9; produção de leite = 25 ± 3,7 kg/d e 19 ± 2,5 kg/d; PC = 553 ± 11 kg e 444 ± 14 kg, respectivamente), foram usadas em um delineamento em blocos ao acaso para o ensaio de desempenho e três vacas canuladas no rúmen foram utilizadas em um quadrado latino 3 x 3 replicado, para o ensaio que avaliou os parâmetros ruminais. As vacas foram alimentadas com silagem de milho ad libitum e com fornecimento individualizado de 9 kg de concentrado, contendo sais de cálcio de ácidos graxos do óleo de palma (CSPO) associados ou não à lisolecitina (1% de Lysoforte®, Kemin Industries, Inc. com 98% de Lisolecitina). O período experimental teve duração de 90 dias, iniciando entre a terceira e quarta semana após o parto. Os tratamentos foram controle (concentrado com 400g de CSPO sem Lisolecitina (CSPO-400); e concentrado com três níveis de CSPO com Lisolecitina a 1% (CSPOL), 280g, 340g e 400g. Os contrastes pré-planejados foram elaborados para determinar o efeito da suplementação com Lisolecitina (Lyso), comparando ambos os tratamentos com 400g de suplementação de CSPO e efeito linear (L) ou quadrático (Q) do aumento da inclusão de CSPO em presença de Lisolecitina no experimento de desempenho e efeito da Lisolecitina (Lyso) e do aumento CSPO em presença de Lisolecitina (de 280g para 400g de CSPOL) (D), no experimento que avaliou os parâmetros ruminais. O consumo de matéria seca, o consumo de nutrientes e a digestibilidade aparente desses nutrientes foram medidos duas vezes durante o período experimental (35 DEL e 65 DEL). Essas variáveis não foram diferentes entre os tratamentos CSPO e CSPOL-400. Os níveis de CSPOL tenderam a causar diminuição linear no consumo de MS e MO, e aumento linear no consumo de AG e na digestibilidade aparente de AG. Não houve efeitos do tratamento na produção de leite, componentes do leite e produção acumulada de leite. O tratamento com CSPOL-400 tendeu a causar diminuição no teor de gordura do leite e aumento no NUL em comparação com CSPO-400, enquanto os níveis de CSPOL causaram diminuição linear no teor de gordura do leite e no teor de NUL. O peso corporal (PC) da vaca, as alterações do PC e o ECC não foram afetados pela alimentação com Lisolecitina e níveis de CSPOL. A alimentação de Lisolecitina para vacas leiteiras alimentadas com 400 g de gordura inerte não teve efeito sobre a ingestão de energia, produção de energia, balanço de energia e eficiência de utilização de energia. Durante o período de tratamento, não houve efeito da Lisolecitina nas concentrações plasmáticas de proteína total, insulina NEFA e ureia. No entanto, a suplementação com Lisolecitina aumentou a concentração de glicose no plasma. Os níveis de CSPOL causaram aumento linear na concentração de glicose no plasma. A alimentação de Lisolecitina para vacas em lactação alimentadas com 400g de gordura inerte não afetou o consumo e a digestibilidade da MS e nutrientes, no pH ruminal, AGV total, proporções molares de acetato, butirato e isobutirato, na relação C2:C3 e no NH_3-N ruminal concentração, mas tendeu a aumentar o propionato ruminal e a aumentar o valerato ruminal. Concluímos que a Lisolecitina não teve efeito sobre a ingestão de nutrientes e digestibilidade no trato total, perfil metabólico-hormonal, partição de energia, produção de leite e na maioria dos componentes do leite, PC e ECC de vacas no terço inicial de lactação alimentadas com 400g de CSPO. Além disso, em presença de Lisolecitina, não houve melhora no desempenho das vacas com o aumento da suplementação com gordura de palma de 280 a 400g/dia.

Palavras-chave: Emulsificante, Lisolecitina, Gordura do leite, Suplementação com gordura

ABSTRACT

Performance and metabolism of early lactation dairy cows receiving doses of calcium salts of palm oil supplemented or not with Lysolecithin

The objectives were to evaluate the effects of supplementation with doses of calcium salts of palm oil with or without Lysolecithin on production responses, nutrient digestibility, energy intake and balance, metabolic responses, and ruminal responses of early lactation dairy cows on dry lot. Forty-four early lactation Holstein cows (Holstein or ¾ Holstein ¼ Jersey), 28 multiparous and 16 primiparous (average ± SD at the beginning of the experiment; DIM = 20 ± 4 and 20 ± 9; milk yield = 25 ± 3.7 kg and 19 ± 2.5 kg; BW = 553 ± 11 kg and 444 ± 14 kg, respectively), were used in a randomized complete block design in the performance trial and three ruminal cannulated cows in a replicated 3 x 3 Latin square design, in the metabolism trial. Cows were fed corn silage ad libitum plus 9 kg of concentrate (as fed), provided individually, containing calcium salts of palm oil (CSPO) associated or not with Lysolecithin (1% of Lysoforte®, Kemin Industries, Inc. with 98% of Lysolecithin). The trial lasted 90 days and started between week 3 and 4 postpartum. The treatments were control (concentrate with 400g of CSPO without Lysolecithin (CSPO-400); and concentrates with three levels of CSPO with 1% Lysolecithin (CSPOL), 280g, 340g and 400g. Preplanned contrasts were made to determine the effects of Lysolecithin (Lyso), comparing the treatments with 400g of CSPO supplementation, and linear (L) or quadratic (Q) effect of increasing CSPO with Lysolecithin (280, 340 and 400g of CSPOL) in the performance trial and effect of Lysolecithin (Lyso) and effect of the CSPO with Lysolecithin increase (280g to 400g of CSPOL); (D), in the metabolism trial. For the performance trial, dry matter intake, intake of dietary nutrients, and apparent digestibility of nutrients were measured twice during the experimental period (at 35 and 65 DIM). These variables were not different between treatments CSPO and CSPOL-400. The levels of CSPOL tended to cause a linear decrease on the intakes of DM and OM, and a linear increase on the intake and apparent digestibility of FA. There were no treatment effects on yields of milk, milk components and on cumulative milk yield. The treatment with CSPOL-400 tended to cause a decrease on milk fat content, and an increase on MUN compared with CSPO-400, while levels of CSPOL caused a linear decrease on milk fat content and on MUN content. Cow BW, BW changes and BCS were not affected by feeding Lysolecithin and levels of CSPOL. Feeding Lysolecithin for dairy cows fed 400 g of inert fat had no effect on energy intake, energy output, energy balance and efficiency of energy utilization. During the treatment period, there was no effect of Lysolecithin on plasma concentrations of total protein, insulin, NEFA and urea. However, supplementing Lysolecithin increased plasma glucose concentration. Levels of CSPOL caused a linear increase on plasma glucose concentration. For the metabolism trial, feeding Lysolecithin to lactating dairy cows fed 400g of inert fat had no effect on intake and digestibility of DM and nutrients, on rumen pH, total VFA, molar proportions of acetate, butyrate and isobutyrate, on C2:C3 ratio and on rumen NH3-N concentration, but tended to increase rumen propionate and increased rumen valerate. We conclude that the Lysolecithin had no effect on nutrient intake and total-tract digestibility, metabolic-hormonal profile, energy partitioning, milk yield and on most milk component contents, BW and BCS of early lactating cows fed 400g of CSPO. In addition, in the presence of Lysolicithin, increasing CSPO supplementation from 280 to 400g did not improve cow performance.

Keywords: Emulsifier, Lysolecithin, Milk fat, Fat supplementation

1. INTRODUCTION

The increase in milk production per cow results in high metabolic demand by the animals (Loften et al., 2014). This situation is more serious during in the postpartum period when the increase in milk yield is high and DMI is low (NRC, 2001), resulting in a negative energy balance (NEB), (Grummer, 1995; Goff and Horst, 1997; Herdt, 2000).

As a strategy to reduce the effects of NEB, increasing the energy density of the diets fed to cows in the postpartum period by feeding lipids is a broadly explored alternative (Palmquist and Mattos, 2006). Rabiee et al. (2012) compiled data from 200 articles, and demonstrated by a meta-analysis that the incorporation of fatty acids (FA) in the diet of lactating dairy cows increased the milk yield and decreased DMI, a fact that allowed the authors to conclude that the FA infusion improves the productive efficiency.

The supplementation period is important to evaluate the FA supplementation, in a meta-analysis by Onetti and Grummer (2004), was observed that fat supplementation increased milk yield and milk fat yield when fed to cows during early lactation (<120 DIM), but it had no effect in mid-lactation (>120 DIM) dairy cows. Additionally, fat supplementation during the immediate postpartum period (1-24 DIM) did not affect milk yield, but during the period of peak of lactation (25-65 DIM) fat increased milk yield consistently (de Souza et al., 2019).

However, the use of dietary FA, depending on the source, can result in negative effects on ruminal fermentation (Jenkins, 1993; Doreau and Ferlay, 1995; Nagaraja et al., 1997; Palmquist and Mattos, 2006). Mosley et al. (2007) reported that, a great number of studies have been developed to allow high dietary inclusion levels of FA, without the negative impacts on rumen fermentation.

One alternative is to use saturated FA. Weiss and Pinos-Rodriguez (2009) demonstrated that high production cows supplemented with 2.25% of free FA (diet dry basis), increased milk yield by 2.60 kg. Harvatine and Allen (2006a) reported a linear increase in milk production of cows supplemented with saturated FA compared to cows supplemented with UFA. Other studies have also demonstrated the positive effects of saturated FA in the performance of lactating dairy cows (Mosley et al., 2007; Lock et al., 2013; Piantoni et al., 2013; Mathews et al., 2016; de Souza et al., 2018).

Another alternative to avoid the negative effects of unsaturated fat supplementation is the use of calcium salts, produced from the hydrolysis of triglycerides and the subsequent association of different FA with calcium, forming salts with a high melting point, stable in a pH greater than 6.0 and therefore inert in the rumen (Palmquist and Jenkins, 2017). These calcium salts, however, dissociated in the low abomasum pH, (Loften and Cornelius, 2004). The dissociation of calcium salts depends on the source used; normally, saturated FA provides a high stability at the normal rumen pH of a dairy cow after feeding, while calcium salts of unsaturated FA have a low stability (Palmquist and Jenkins, 2017).

De Souza et al. (2017), observed that compared with calcium salts of soybean FA (CSSO), calcium salts of palm FA (CSPO) increased milk yield, milk component yields and nutrient digestibility of early-lactation dairy cows. The results found by de Souza et al. (2017) can be explained by the high UFA load present in the CSSO supplement, which favored ruminal dissociation. Additionally, CSSO fed cows had milk fat depression, reinforcing that the a high dissociation occurred in the rumen, exposing the microorganisms to UFA and inducing the formation of biohydrogenation intermediates such as CLA *trans*-10 *cis*-12, which acts on the mammary gland inhibiting the expression of enzymes associated with de novo synthesis (Baumgard et al., 2002). However, CSPO have more saturated FA than CSSO in their composition, thus they are more stable in the rumen (Sukhija and Palmquist, 1990).

Due to the common practice of fat supplementation for lactating dairy cows, the use of exogenous emulsifiers has been studied (Jenkins, 1993; Nagaraja et al., 1997; Wettstein et al., 2000). Lecithin is a complex mixture of different phospholipids and triglyceride residues obtained during the processing of oilseed grains (Seidman et al., 2002). These mixtures can be hydrolyzed and mixed to obtain an additive with emulsifying properties (FEEDAP, 2016). Lysolecithin is an example, which is naturally formed in the lumen by the small intestine of ruminants by the enzymatic hydrolysis of lecithin by phospholipase A2 secreted by the pancreas (Noble, 1978; Rico et al., 2017a), and it is also produced the rumen after phospholipid degradation but in a lesser extent (Dawson, 1959).

Lysolecithin is important for the absorption of FA from the diet because it is required for the formation of micelles (Davis, 1990). As Lysolecithin has a greater emulsifying capacity, it produces a micellar structure with droplets of FA smaller than the those from lecithin for example (Reynier et al., 1985), and they are more stable in the small intestine, allowing the absorption of fat substances by enterocytes on intestinal epithelium, where the FA are re-esterified to triglycerides and packed in chylomicrons for transportation throughout the bloodstream (Bauman and Lock, 2006).

In rats, reduced content of Lysolecithin in the intestine reduced the absorptive surface area in the intestine (da Silva et al., 2015), which impairs the secretion of chylomicron into lymph (Takahashi et al., 1982). Thereby, this suggests that Lysolecithin enhances the FA absorptive capacity in the gastrointestinal tract, particularly of lipids. In poultry diets, Lysolecithin has increased the productive performance of animals due the greater efficiency in the use of basal FA (Siyal et al., 2017; Wealleans et al., 2020). The use of Lysolecithin for this purpose in monogastric is widespread. Some studies registered an increase in feed efficiency and egg weight (Han et al., 2010). Broilers in the initial growth phase had increased weight gain due to the greater digestibility of FA (Zhang et al., 2011), and improved feed efficiency (Khonyoung et al., 2015; Zampiga et al., 2016; Wealleans et al., 2020).

In ruminants, there are few studies evaluating the effects of dietary phospholipids. Wettstein et al. (2001) evaluated the effect of different sources of hydrolyzed or not lecithin, replacing 25% of CSPO in the diets of lactating cows and did not observe effects milk production, milk composition, and apparent dry matter digestibility. However, in studies with sheep, increased fiber digestibility was reported (Jenkins and Fotouhi, 1985; Yoon et al., 1986). Feeding levels of CSPO to early lactation grazing cows, (negative control, 200g, 400g, and 600g as-fed), had a positive linear effect on FA intake and a positive quadratic effect on FA digestibility, with the maximum value of FA digestibility at 400g of CSPO (Santos Neto, 2020). The total tract digestibilities of FA from early lactation grazing cows fed 0, 200, 400 and 600g of CSPO were respectively 74.2, 76.9, 79.7 and 77.2%. Due to the theoretical potential of using supplemental FA sources in association with emulsifiers to optimize the absorption of FA with an increase in FA digestibility, it is justified to conduct studies to evaluate the effects of this combination on the physiological, metabolic and productive responses of lactating dairy cows.

Therefore, the objective of our study was to evaluate the effects of CSPO supplementation with Lysolecithin on the yields of milk and milk components, metabolic parameters, and nutrient digestibility of early-lactation dairy cows. We hypothesized that a) Supplementing Lysolecithin to early lactating cows fed 400 g of CSPO would increase FA digestibility and cow performance; b) early lactation cows do not respond to increasing levels of CSPO supplementation in the presence of Lysolecithin.

2. MATERIALS AND METHODS

The study was conducted in Piracicaba, SP, Brazil (22º42´S, 47º38´W and 546 a.s.l), at the experimental farm of the University of São Paulo, campus Luiz de Queiroz, College of Agriculture (USP/ESALQ). Animals involved in this experiment were cared for according to the guidelines of the Institutional Animal Care and Ethical Committee for Animal Research (CEUA - USP/ESALQ). The committee reviewed and approved all procedures carried out in this study (protocol number, 2018.5.925.11.6).

2.1. Performance trial

2.1.1. Date, Animal Housing and Care

The performance trial began on April 17, 2018 and ended on December 21, 2018. Experimental cows from the University dairy grazing herd were housed as a single group in a dry lot. The paddock was 60 by 25 m with 40 meters linear bunk space for ad libitum corn silage feeding, with free access to natural shade and fresh water. The cows remained during the entire experimental period in the paddock, leaving it only to be fed their individual dose of concentrate at 6:00 and 18:00 h and then milked at 7:00 and 19:00 h twice a day. The University dairy farm reproductive and health protocols were maintained during this study.

2.1.2. Animals, Treatment Diets and Experimental Design

Forty-four early lactation cows (Holstein or ¾ Holstein ¼ Jersey), 28 multiparous and 16 primiparous (average ± SD at the beginning of the experiment; DIM = 20 ± 4 and 20 ± 9; milk yield = 25 ± 3.7 kg and 19 ± 2.5 kg; BW = 553 ± 11 kg and 444 ± 14 kg, respectively), were used in a randomized complete block design to evaluate cow performance, blood parameters and diet digestibility.

Cows were blocked by parity (primiparous vs. multiparous), date of parturition (maximum of 14 days of variation within each block) and the average milk production during the first 3 weeks postpartum, and during this period milk samples were taken for analysis and milk yield and composition were used as a covariate. During this preliminary period cows were fed corn silage ad libitum plus 7 kg (as fed basis) of a basal concentrate balanced according to NRC (2001) and composed of ground corn, soybean meal, sodium bicarbonate and mineral plus vitamin supplement. During the treatment period, from week 4 to 17 postpartum, all cows remained confined in a dry lot as a single group, receiving corn silage ad libitum as the roughage source. The experimental concentrates containing CSPO (CSPO; 85.2% FA with 40.4% C16:0; 3.9% C18:0; 31.8% C18:1 cis-9; 6.5% C18:2 cis-9, cis-12; and others <4% each) or doses of CSPO associated with 1% of Lysolecithin (CSPOL). The treatments (Table 1) were a concentrate (9 kg as fed basis per cow daily) containing 400 g of CSPO (CSPO-400), or the same amounts of concentrates containing three levels of CSPO plus Lysolecithin (CSPOL): 280 g (CSPOL-280), 340 g (CSPOL-340), or 400 g (CSPOL-400). An equivalent amount of the basal concentrate was replaced by the respective amount of CSPO or CSPOL of every treatment and mixed daily and individually, just before feeding for every cow. The

experimental cows were contented in a tie-stall system and fed the experimental concentrates one hour before the morning and afternoon milking.

The CSPO (EnerFat®) and the Lysolecithin (Lysoforte eXtend®, with 98% of Lysolecithin) were from Kemin Industries, Inc..

During the experimental period, one primiparous cow with 50 DIM was removed from the experiment because of an accidental injury.

2.1.3. Data and Sample Collection

The milk yield was recorded daily (flow meter, DeLaval F17® - DeLaval, Tumba, Sweden), cows were milked twice a day throughout the entire experiment (7:00 and 19:00 h), and milk samples from individual cows were collected every Tuesday afternoon (maintained refrigerated) and Wednesday morning, preserved with a bronopol pill, composited by day (50:50 afternoon and morning milking), and sent after the morning milking to the laboratory for composition analysis. All cows were weighed and body condition was scored weekly by 3 trained investigators on a 5-point scale (1 = thin and 5 = fat), as described by Wildman et al. (1982), with 0.25 increments (Edmonson et al., 1989; Chalupa et al., 1996). Samples of all diet ingredients (0.5 kg) were collected weekly for chemical composition analysis and stored at −20°C.

Corn silage intake and total tract nutrient digestibility were measured twice during the experimental period (35 and 65 DIM) from determination of total fecal excretion and feed indigestibility. To determine fecal excretion, titanium dioxide (TiO_2) was dosed twice a day (20 g/cow per day) before each milking for 15 d. The titanium dioxide was top dressed individually on the experimental concentrates, after contention of cows in a tie-stall system, one hour before each milking. Fecal samples were collected at each time point from the rectum of individual cows, after morning and afternoon milking from the last 5 d, and samples were composited by cow and period, and immediately frozen at −20° (de Souza et al., 2014), after the samples were dried in a forced-air oven at 55ºC for 72 h and ground through a 1-mm screen (Willey-type mill, MA-680; Marconi Ltda., Piracicaba, Brazil).

During the treatment period, blood samples (~15 mL) were collected by venipuncture of jugular vessels within 1 h after concentrate feeding on d 35 and 65 DIM. Blood was collected into two vacuum tubes, one containing anticoagulant and the other containing a glycolytic inhibitor, and stored on ice until centrifugation at 2000 × g for 15 min at 4°C, the plasma was transferred into microcentrifuge tubes and stored at −20°C.

2.1.4. Sample Analysis and Calculations

Milk samples were analyzed for fat, protein, lactose, total solids, and MUN using infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Yields of 3.5% FCM and energy-corrected milk, were calculated using average weekly milk yield and component concentrations, according to equation of NRC (2001), as follow: 3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)]; ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)].

Mean daily BW change (kg/d) was calculated for each cow within period, and Energy output in body reserves (Mcal/d) was estimated according to NRC (2001). Body reserves output (Mcal/d) = [(2.88 + 1.036 x BCS) x

ΔBW], where BCS was the average BCS and ΔBW was BW change measured during the weeks relative to 35 and 65 days postpartum.

Composite feed ingredients and fecal samples were thawed, dried at 55°C in a forced-air oven for 72 h and ground through a 1-mm screen (Willey-type mill, MA-680; Marconi Ltda., Piracicaba, Brazil). The DM was determined by drying samples in an oven at 105°C for 24 h, ash (AOAC, 1990), total N content by the Dumas combustion method using N analyzer (Leco FP-2000 N Analyzer; Leco Instruments Inc., St. Joseph, MI), NDF (Van Soest et al., 1991) with sodium sulfite and heat-stable α-amylase for all feed and fecal samples in an Ankom-200 (Ankom Tech Corp., Fairport, NY), ADF (Goering and Van Soest, 1970), in an Ankom-200 (Ankom Tech. Corp., Fairport, NY) and lignin (AOAC, 2005), Ether Extract (AOAC, 2005), in the Ankom® XT15 analyzer (Ankom Tech Corp., Fairport, NY), we used the NRC (2001) equation to convert EE into total FA by the following equation: total FA (% of DM) = EE (% of DM) - 1.

Forage intake was estimated from feed indigestibility and total fecal excretion. Indigestible NDF was estimated as NDF after a 240-h in situ incubation (Huhtanen et al., 1994). Fecal samples were analyzed for titanium concentration according to Myers et al. (2004). and total fecal excretion calculation, fecal excretion coming from the concentrate, and forage intake were calculated according to de Souza et al. (2014).

Energy values were calculated based on nutrient digestibility (d). The values were calculated using equations from (NRC, 2001), as follow: For concentrate and silage: DE (Mcal/kg) = (dNFC x 0.042) + (dNDF x 0.042) + (dCP x 0.056) + ((dFA/2.25) x 0.094)) - 0.3; ME (Mcal/kg) = ((1.01 x (ED) – 0.45) + 0.046 x (EE -3); NEL (Mcal/kg) = 0.703 X ME - 0.19 + ([(0.097 X ME + 0.19)/97] X [EE - 3]); For FA supplement: DE (Mcal/kg) = 9.4 x (dFA x 0.09 x (EE/100) + (4.3 x (EE/100)); ME (Mcal/kg) = DE (Mcal/kg); NEL (Mcal/kg) = 0.8 x ME (Mcal/kg). The intakes of DE, ME and NEL were calculated by multiplying DMI by the respective energy contents.

Energy outputs were calculated based on milk yield and composition from the respective days of DMI measurement (35 and 65 days postpartum). Energy balance was calculated as NEL intake − NEL milk – NEL maintenance, according to the NRC (2001), as follow: NEL milk (Mcal/d) = Milk yield (kg) × (0.0929 × fat % + 0.0563 × true protein % + 0.0395 × lactose %); NEL maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg)$^{0.75}$. In addition, the partitioning of energy intake to milk, maintenance and to body reserves were calculated. Body reserves output (Mcal/d) = [(2.88 + 1.036 x BCS) x ΔBW].

Blood plasma was analyzed for NEFA, total protein, glucose, urea, and insulin hormone concentrations, using ELISA methods and determined by Multiskam MS ELISA (Labsystems, Helsinki, Finland).

2.1.5. Statistical Analyses

To production and metabolic-hormonal profile data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to the following model with repeated measures:

Yijklm = μ+ βi + Nj + Єij + Tk + C(N)l(j) + NTjk + Cov + Єijkl, where Yijkl = the dependent variable, μ = the overall mean, βi = the random effect of block (i = 1 to 11), Nj = the fixed effect of treatment (j = CSPO or CSPOL), Єij = the residual error, Tk = the fixed effect of time, C(N)l(j) = the random effect of cow nested in treatment, NTjk = the fixed effect of interaction between treatment and time, Cov = the covariate, and Єijkl = the residual error. All repeated measures were included with the covariance structure of [AR (1)] according to the lowest Bayesian information criterion. Cumulative milk yield, nutrient digestibility, and energy partitioning data were analyzed using a reduced model without the main effect of time and interactions between time and treatment.

Planned contrast statement was used to evaluate the overall effect of 400g CSPOL versus 400g CSPO in the treatment period, and effect of increasing dose of CSPOL. Significance statistical were declared at $P \leq 0.05$. Differences between treatments with $0.05 < P < 0.10$ were considered as a tendency toward significance.

2.2. Metabolism trial

2.2.1. Date, Animal Housing and Care

The metabolism trial with cows fitted with rumen canula lasted from March 16, 2019 to July 14, 2019. Experimental cows from the University dairy grazing herd were housed as a single group in a dry lot. The paddock was 10 by 25 m with 10 meters linear bunk space for ad libitum corn silage feeding, with free access to natural shade and fresh water. The cows remained during the entire experimental period in the paddock, leaving it only to be fed their individual dose of concentrate at 6:00 and 18:00 h and then milked at 7:00 and 19:00 h twice a day. The University dairy farm reproductive and health protocols were maintained during this study.

2.2.2. Animals, Treatment Diets and Experimental Design

Three multiparous lactation ruminally cannulated Holstein or ¾ Holstein ¼ Jersey cows, (average ± SD at the beginning of the experiment; DIM = 163 ± 20; milk yield = 14 ± 1.6 kg; BW = 546 ± 13 kg), allocated in a replicated, balanced and not contemporary 3 × 3 Latin square were used to evaluate diet digestibility and rumen fermentation. The 3 x 3 replicated Latin Square was composed of 3 periods of 20 days each.

During the treatment period, all cows remained confined in a dry lot as a single group, receiving corn silage ad libitum as the roughage source. The treatments were a concentrate (9 kg as fed basis per cow daily) containing 400g of CSPO, or the same amount of concentrates containing two levels of CSPOL: 280 g, or 400 g. The respective CSPO or CSPOL doses were mixed daily and individually to a common basal concentrate formulation, just before feeding for every cow, after contention of cows in a tie-stall system before each milking. The basal concentrate was balanced according to NRC (2001) and was composed of ground corn, soybean meal, sodium bicarbonate and mineral supplement and then mixed with the respective dose of CSPO or CSPOL (Table 1).

The CSPO (EnerFat®) and the Lysolecithin (Lysoforte eXtend®, witch 98% of Lysolecithin) were from Kemin Industries, Inc..

2.2.3. Data and Sample Collection

Samples of all diet ingredients (0.5 kg) were collected during each experimental period of the replicated squares for chemical composition analysis and stored at −20°C. Corn silage intake, DMI and total tract nutrient digestibility were measured during each experimental period of the replicated squares, from determination of total fecal excretion and feed indigestibility. To determine fecal excretion, titanium dioxide (TiO_2) was dosed twice a day (20 g/cow per day) after each milking for 15 d (day 5 to 15 for adaptation and day 16 to 20 for fecal collection) during the 3 periods of the Latin Squares. Fecal samples were collected at each time point from the rectum of

individual cows, after morning and afternoon milking and samples were composited by cow and period, and immediately frozen at −20°C (de Souza et al., 2014).

Ruminal samples (approximately 50 ml of rumen fluid), were collected from individual cows at 0, 2, 4, 6, 8, 12, 18 and 24 h after morning concentrate feeding on d 20 and 21 (Broderick and Reynal, 2009) from 4 different locations in the rumen, with the support of a probe (cranial ventral, central ventral, central dorsal, and caudal ventral), mixed and then a subsample was taken. The rumen content subsample was filtered into 4 layers of cheesecloth, its pH was measured immediately (Broderick and Reynal, 2009), using a portable pH meter (Digimed Model DM22, Digicrom Analitica LTDA., SP, Brazil). Aliquots of ruminal fluid were stored in microcentrifuge tubes and frozen at −20°C, until processing.

2.2.4. Sample Analysis and Calculations

In preparation for analysis feed and fecal samples were thawed, dried in a forced-air oven at 55ºC for 72 h and ground through a 1-mm screen (Willey-type mill, MA-680; Marconi Ltda., Piracicaba, Brazil).

Composite feed ingredients, fecal samples, forage intake, feed indigestibility, nutrient digestibility, energy values, and energy output were analyzed and estimated as described for the performance study. All samples of ruminal fluid were centrifuged (15.000 x g, 4ºC, 30 min), and analyzed for molar proportions VFA (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, as well as the acetate:propionate ratio, and total VFA), according to Ferreira et al. (2016), using gas-liquid chromatography (Palmquist and Conrad, 1971), and for ammonia nitrogen (Chaney and Marbach, 1962).

2.2.5. Statistical Analyses

Data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to the following model:

$Yijk = \mu + Ci + Sj + P(S)k(l) + Tl + eijkl$, where Yijk = the dependent variable, μ = the overall mean, Ci = random effect of cow (i = 1 to 3), Sj = the fixed effect of treatment (j = 1 to 3), P(S)k(l) = the fixed effect of period nested within square (k = 1 to 6), Tl = the fixed effect of square (l = 1 to 2), and eijkl = the residual error.

Planned contrast statement was used to evaluate the overall effect of 400g CSPOL versus 400g CSPO in the treatment period, and effect of increasing dose of CSPOL. Significance statistical were declared at $P \leq 0.05$. Differences between treatments with $0.05 < P < 0.10$ were considered as a tendency toward significance.

3. RESULTS

3.1. Nutrient Intake and Total-Tract Digestibility

There were no interactions ($P > 0.05$) between treatment and time for any variable evaluated (Table 3).

Dry matter intake, intake of dietary nutrients, and apparent digestibility of these nutrients were measured twice during the experimental period (35 DIM and 65 DIM). These variables were not different between treatments CSPO and CSPOL-400 ($P > 0.05$, Table 3). However, levels of CSPOL tended to cause a linear decrease on the intakes of DM ($P = 0.07$) and OM ($P = 0.08$), and a linear increase on the intake of FA ($P < 0.01$) and on the apparent digestibility of FA ($P < 0.01$, Table 3).

3.2. Production Responses

There were no interactions ($P > 0.05$) between treatment and week of lactation for any variable evaluated (Table 2).

There were no treatment effects ($P > 0.05$) on yields of milk ($P = 0.20$; Figure 1), fat ($P = 0.95$), protein ($P = 0.82$), casein ($P = 0.14$), lactose ($P = 0.36$), 3.5% FCM ($P = 0.97$), ECM ($P = 0.90$), and on cumulative milk yield ($P = 0.36$). However, the treatment with CSPOL-400 tended to cause a decrease on milk fat content ($P = 0.06$), and an increase on MUN ($P = 0.07$) compared with CSPO-400, while levels of CSPOL caused a linear decrease on milk fat content ($P = 0.01$) and on MUN content ($P = 0.05$) (Table 2).

Cow BW, BW changes and BCS were not affected ($P > 0.05$) by feeding Lysolecithin and levels of CSPOL.

There was an effect ($P \leq 0.05$) of week of lactation on yields of milk ($P < 0.01$), fat ($P = 0.05$), protein ($P = 0.02$), casein ($P < 0.01$), lactose ($P < 0.01$), 3.5% FCM ($P = 0.02$), ECM ($P = 0.02$) and on contents of milk lactose ($P = 0.02$; Table 2; Figure 2). A trend was observed for the contents of milk fat ($P = 0.07$) and milk protein ($P = 0.07$). Cow BW and BCS also were affected ($P<0.01$) by week of lactation (Table 2; Figure 2).

3.3. Energy Outputs

There were no interactions ($P > 0.05$) between treatment and period (35 DIM versus 65 DIM, Table 4).

Feeding Lysolecithin for dairy cows fed 400 g of inert fat (CSPOL-400 x CSPO-400) had no effect ($P > 0.05$) on energy intake, energy output, energy balance and efficiency of energy utilization (Table 4).

We observed that energy outputs to body reserves ($P<0.01$), energy balance ($P=0.01$), and efficiency of conversion of DE to NEL production ($P=0.02$) were affected by period (35 DIM versus 65 DIM), (Table 4; Figure 3). Energy used for body reserves ($P < 0.01$) and energy balance ($P = 0.01$) were greater at 65 than at 35 DIM, while the efficiency of conversion of DE to NEL production was greater ($P = 0.02$) for cows at 35 DIM than at 65.

3.4. Blood Metabolites

There were no interactions (P > 0.05) between treatment and period (35 DIM versus 65 DIM, Table 5).

During the treatment period, there was no effect of Lysolecithin (CSPO-400 versus CSPOL-400) on plasma concentrations of total protein (P = 0.83), insulin (P = 0.58) NEFA (P = 0.67) and urea (P = 0.39). However, supplementing Lysolecithin increased plasma glucose concentration (P = 0.03). Levels of CSPOL caused a linear increase on plasma glucose concentration (P = 0.05).

Plasma concentrations of protein (P = 0.01) and insulin (P = 0.05) were affected by period (35 DIM versus 65 DIM) (Table 4; Figure 4), with both metabolites concentrations greater at 65 than at 35 DIM.

3.5. Ruminal pH and Fermentation

Feeding Lysolecithin to lactating dairy cows fed 400g of inert fat (CSPOL-400 vs CSPO-400) had no effect (P > 0.05) on intake (P > 0.05) and digestibility of DM and nutrients (P > 0.05), on rumen pH (P = 0.31), total VFA (P = 0.52), molar proportions of acetate (P = 0.15), butyrate (P = 0.20) and isobutyrate (P = 0.14), on C2:C3 ratio (P = 0.45) and on rumen NH3-N concentration (P = 0.19), but tended (P = 0.09) to increase rumen propionate and increased (P = 0.05) rumen valerate (Table 6). Compared with CSPOL-280 feeding CSPOL-400 increased (P = 0.03) rumen valerate.

4. DISCUSSION

More recently, the effects of FA supplements on digestibility responses of dairy cows have received renewed attention, because some of them have reported that the digestibility of FA usually decreases as FA intake increases (Palmquist, 1994; Piantoni et al., 2013; Boerman et al., 2015a). Weiss and Tebble (2019) summarized a 20 years data set (207 observations) from their lab and conducted a Lucas test on fatty acid digestibility. They obtained true digestibility values to FA between 73% and 75%. These low digestibility values may indicate a potential limitation in Lysolecithin secretion reducing the flux of FA into micelles (Drackley, 2000; Boerman et al., 2015a) or due a possible saturation of absorptive sites in the intestine (Glasser et al., 2008). The present experiment evaluated the effects of levels of calcium salts of FA from palm oil with Lysolecithin supplementation. We expected, that feeding Lysolecithin would increase the total digestibility of the lipids, and this would enable a reduction in the supply of lipids with similar performance.

We observed that the association between Lysolecithin and CSPO did not affect the total tract digestibility of FA in both performance and metabolism trials. Previous studies reported that fat digestibility is affected by the profile of FA reaching the intestine (Doreau and Chilliard, 1997; Boerman et al., 2015a). Results have indicated that *cis*-9 C18:1 has been greater digestibility than C16:0 and C18:0 (Boerman et al., 2015a), and *cis*-9 C18:1 has been suggested as having amphiphilic properties (Moate et al., 2004). The lipid source fed in our experiment had 31.8% *cis*-9 C18:1 in its composition, thus contributing to a greater digestibility of the FA in the intestine. This is supported by Freeman (1969), who examined the amphiphilic properties of polar lipid solutes and reported that *cis*-9 C18:1 had a positive effect on the micellar solubility. In addition, C16:0 has a greater digestibility than C18:0. Feeding C18:0-enriched FA supplements has been shown to decrease FA digestibility in dairy cows (Piantoni et al., 2015; Boerman et al., 2017) and sheep (Toral et al., 2016). Boerman et al. (2017) suggested that the lower digestibility of C18:0-enriched FA supplements may be related to their higher melting point compared with other supplements. Similarly, Chamberlain and DePeters (2017) observed that increasing the proportion of C18:0 to C16:0 in supplemental fat (2% of diet DM) reduced total FA digestibility in dairy cows. Therefore, it is possible that the calcium salts of palm oil used in the present study, 31.8% C18:1 and 40.2% C16:0 did not impose a challenge for intestinal FA digestion. In the present study total tract digestibility of FA varied from 79.5 to 83.6% in the performance trial and from 75.9 to 78.9% in the metabolism trial, similarly, Purushothaman et al. (2008), feeding levels of CSPO (concentrate mixture containing 0, 2, 4, and 6% of CSPO on DM basis) to low-milk yield mid lactation dairy cows (13 to 15kg of milk daily) reported values of FA digestibility from 84.7 to 88.6%. However, these values are consistently higher than the true digestibility values from 73 to 75% for FA reported by Weiss and Tebble (2019).

In a previous study from our Lab, feeding levels of CSPO, (negative control, 200g, 400g, and 600g as-fed), had a positive linear effect on FA intake and a positive quadratic effect on FA digestibility, with the maximum value of FA digestibility at 400g CSPO (Santos Neto, 2020). The total tract digestibilities of FA from early lactation grazing cows fed 0, 200, 400 and 600g of CSPO were respectively 74.2, 76.9, 79.7 and 77.2%. This result suggests that a potential limitation of Lysolecithin for fat digestion in the small intestine can occurs at higher levels than those used in our trial. Similarly, in the performance trial of the present study, increasing CSPOL levels from 280 to 400g also increased, total tract FA digestibility.

In nonruminants, the lysophospholipid fed appears to interact with the type of FA. Jansen et al. (2015) evaluated the effects of lysophosphatidylcholine on two FA sources, soybean oil (83% unsaturated fatty acids) and

pig lard (59% unsaturated fatty acids). They observed that Lysolecithin supplementation in broiler diets showed a significant interaction with the fat type, both in vitro and in vivo. The Lysolecithin significantly increased the digestibility of pig lard treatments ($P < 0.01$), but did not increase the digestibility of soybean oil ($P > 0.05$), suggesting that Lysolecithin is able to improve the digestibility of FA supplement, especially when they have more saturated FA in their composition. Further studies are needed to understand the effects of Lysolecithin fed with specific enriched FA-supplements to ruminants.

Feeding Lysolecithin to cows fed 400g of calcium salts of palm oil (CSPOL-400 x CSPO-400) did not affect DM digestibility, differently from Lee et al. (2019) who reported that feeding two levels of Lysolecithin (0.05% and 0.075% of dietary DM), to lactating cows tended to decrease apparent digestibility of DM and OM. In our study, we used lower levels of Lysolecithin (0.98% of the fat supplements CSPO-280, CSPO-340 and CSPO-400), with the maximum value around 0.023% of dietary DM for the CSPOL-400.

Interestingly, we observed that feeding CSPOL-400 did not affect DMI compared with CSPO-400. However, a previous study from Rico et al. (2017a) reported that feeding 10g/d Lysolecithin reduced DMI in cows fed lower-NDF dietary (29% NDF) and higher unsaturated FA (2% oil from whole soybeans and soybean oil), however they did not report any effect on cows fed standard fiber (30.5% NDF) and lower fat diet (no added oil). These studies suggest that feeding unprotected Lysolecithin is best for cows fed diets adequate in NDF with limited unsaturated fat. Recently, a study with exogenous emulsifiers (polysorbate-C18:1) reported that a dose of 45 g/d promoted a negative quadratic effect on DMI (de Souza et al., 2020). These authors associated this response with the hypophagic effect that C18:1 has by stimulating the release of gut satiety signals in the gut. The actual reasons why Lysolecithin reduces DMI in some studies but not in ours are unknow and deserve further investigations.

In our trial the Lysolecithin was fed associated with calcium salts of palm FA, source of palmitic acid (40.2% of C16:0). A meta-analysis by Weld and Armentano (2017) reported that each percentage-unit increase of CSPO in lactating dairy cow diets decreased DMI by 0.40 kg. Interesting, these authors observed that CSPO tended to increase NDF digestibility. Other studies have reported the same (Boerman et al., 2017; Rico et al., 2017b; de Souza and Lock, 2019). Possibly, C16:0 may increase the retention time by an increase in cholecystokinin secretion (Piantoni et al., 2013); also, C16:0 may be incorporated into rumen bacterial membranes instead of being synthesized, which would spare ATP and thus would favor bacterial growth and, NDF digestibility (Vlaeminck et al., 2006). In our study, the effect of CSPO supplement may have masked the negative response of Lysolecithin, or the calcium salts may rumen protect Lysolecithin to possible action on the intestine. However, the number of studies that evaluated the response of ruminants to supplementation with lysophospholipids (LPL) is limited (two results are available; Rico et al., 2017a; Lee et al., 2019). This has been examined mostly in nonruminant animals.

We did not observe changes on the yields of milk, fat, protein, lactose, 3.5% FCM, ECM, and cumulative milk. Similarly, Rico et al. (2017a) fed a diet with Lysolecithin (10 g/d) to dairy cows and observed no increase in the milk yield. However, a recent study with exogenous emulsifiers, observed an increase in 3.5% FCM and ECM (de Souza et al., 2020). In addition, Lee et al. (2019) reported an increase of ~2.0 kg/d in the milk yield of cows fed with two levels of LPL (0.05% and 0.075% of dietary DM), compared with a control diet. The results of feeding phospholipids on milk production are inconsistent because the sources of phospholipids and the enzymatic hydrolysis process to produce lysophospholipids, have a great variability (Lee et al., 2019). Another point is that feeding unprotected Lysolecithin or lecithin, may not be the solution to increase FA digestibility in the small intestine, because these supplements may not reach the small intestine. A type of being ruminal protection could be alternative.

We observed that CSPOL-400 tended to increase MUN (P = 0.07) compared with CSPO-400. Lee et al. (2019), observed that feeding 0.075% of Lysolecithin (% of dietary DM) for lactating dairy cows increased N in milk compared with feeding 0.05% of Lysolecithin. These authors hypothesized that dietary N was absorbed in more utilizable forms of N for protein synthesis in the body, because the urinary N decreases. Furthermore, Brautigan et al. (2017) and Chen et al., (2019) recently found that feeding Lysolecithin to chickens potentially increases nutrient absorption due to a positive effect on the expression of the genes involved in intestinal villus length and width, which increased intestinal health.

Previous studies with dairy cows reported that emulsifier supplementation increased milk fat content (Lee et al., 2019). Conversely, Rico et al., (2017a), reported no differences in milk fat content to Lysolecithin supplementation. In our study, however, we observed that Lysolecithin (CSPOL-400 vs CSPO-400) tended to decrease milk fat content. These inconsistent responses may occur due of the type of FA supplied, given that unsaturated FA have higher digestibility than saturated FA (Boerman et al., 2015a). The CSPO used in our study had 40.6% of C16:0 and 37% of C18:1, thus it naturally has a greater digestibility than a source with more than 80% saturated FA, which could contribute to the non-effect observed in our study. Rico et al., (2017a) used a source of unsaturated FA to induce depression in milk fat (MFD). Lee et al. (2019), used hydrolyzed tallow, a saturated FA source (~80% saturated FA; NRC, 2001) that may have contributed to increasing FA digestibility compared to control, because of less digestibility of saturated FA.

As well as in our study, Rico et al. (2017a) did not observe any effect of Lysolecithin on cows BW. However, for broilers, it was observed that Lysolecithin improved feed efficiency, increased BW gain and apparent metabolizable (Wealleans et al., 2020).

The effects of time (35 vs 65 DIM) on yields of milk, fat, protein, lactose, 3.5% FCM, ECM, BW and BCS were expected as cows approach peak of lactation (Chilliard, 1993; García and Holmes, 2001; Hutjens, 2016).

The absence of effect of Lysolecithin on energy intakes, energy outputs, energy balance, and energy efficiencies were expected once DMI, nutrient digestibility and milk yield were not affected by this feed additive. It is interesting to note that in our study, predicting NEL from dietary composition using the NRC (2001) model estimated a NEL intake of 1.69 Mcal/kg DM, whereas our actual calculated NEL intake was 1.77 Mcal/kg DM (an increase in 4.7%). This difference is at least in part associated with the observed increase in NDF and FA digestibility with the use of CSPO comparing with the NRC (2001) model, which is not able to take into account these effects. There is a high variability in nutrient digestibility among cows, and different effects depending on the FA source (de Souza and Lock, 2019). Using energy predictions from dietary composition is inadequate for calculating energy intake and energy balance (Piantoni et al., 2015).

We observed that energy used for body reserves and energy balance was greater at 65 than at 35 DIM, while the efficiency of conversion of DE to NEL production was greater for cows at 35 DIM than at 65. These results agree with previous studies, where the increase in milk production per cow results in high metabolic demand in the peak period (Loften et al., 2014). However, in the post-peak period it was observed a decrease in milk yield with less conversion of DE to NEL production, therefore increasing energy output o body reserves and, energy balance (Chilliard, 1993; García and Holmes, 2001; Hutjens, 2016).

The increase on plasma glucose with Lysolecithin and with fat supplementatioin may be related to an alteration in the glucose requirement by mammary gland (Nichols et al., 2019b), because the FA supplementation with Lysolecithin may have increased the availability of long-chain FA and decreased the de novo FA synthesis by the gland (Grummer and Carroll, 1991; Chilliard, 1993; Hammon et al., 2008). This may have reduced glucose

requirements for oxidative catabolism (Nichols et al., 2019a), increasing glucose in the bloodstream. In contrast, de Souza (2020), did not observe any effect of exogenous emulsifier on plasma glucose concentrations.

In addition, Koster et al. (2017), reported that glucose metabolism can be subdivided into insulin-dependent, influenced by the increase in insulin after a glucose increase, stimulated by insulin-sensitive tissues with an increase in glucose uptake and, insulin-independent, which is not influenced by the increasing insulin concentration. We observed that an increase in glucose did not affect insulin in the bloodstream. This is frequently observed in lactating cows, due the glucose requirement by mammary gland to milk synthesis (Bell and Bauman, 1997; Herdt, 2000).

The positive effect of Lysolecithin (CSPOL-400 vs CSPO-400) on plasma glucose may also be consequence of the greater concentration of ruminal propionate, the major precursor of hepatic glucose in lactating dairy cows (NRC, 2001). The positive effects of Lysolecithin on ruminal propionate was not observed by Lee et al. (2019). They observed a reduction in the proportion of acetate in total VFA with no differences in propionate proportion, resulting in a tendency for decreasing the ratio of acetate to propionate when lysophospholipids where fed to lactating cows. They suggested that the decrease in acetate occurs due to a decrease in NDF digestibility and due to an alteration on the ruminal bacterial profile. However, the association between calcium salts of palm FA and Lysolecithin may be related to modifications in ruminal fermentation.

The positive effect of Lysolecithin on the concentration of valerate was also reported by Lee et. al. (2019), who reported a linear increase in valerate concentration of cows fed 0, 0.05% and 0.075% Lysolecithin in the dietary DM. However, in this study, the increase in valerate concentration was associated with a decrease in NDF digestibility, in contrast to our study where we did not observe a decrease in NDF digestibility. In previous studies the increasing valerate, propionate, butyrate and isovalerate have been associated with greater feed efficiency (Guan et al., 2008; Kruger Ben Shabat et al., 2016). Valerate is precursor of odd chain fatty acids (OCFA), incorporated into ruminal bacteria responsible for increased ruminal propionate (Nasrollahi et al., 2019). In addition, recent research has indicated that an increase in valerate concentration in ruminal fluid, was negatively correlated with milk fat content (Nasrollahi et al., 2019); The autors related valerate with a low ruminal pH (< 5.80) and with an increase in aspartate amino-transferase (AST) activity, which may signalize liver disorders and cause a decrease in milk fat content. We observed a decrease tendency on milk fat content with CSPOL-400, but we did not observe low pH values.

We did not observe effects of Lysolecithin on the total VFA concentration, pH and nutrient digestibility in the ruminal trial. However, the replacement of dietary rumen-protected fat (calcium soaps of palm oil fatty acids) with lecithin (raw, deoiled and deoiled / partially hydrolysed soy lecithin, and raw canola lecithin) did increase in pH and; VFA concentration in dairy cows (Wettstein et al., 2000). In additional, we observed the lowest pH value 2 hours after feeding (6.21) and the highest concentration of VFA 6 hours after feeding. It is necessary to understand the action mode of rumen microbes to develop inert protection technologies to explore the benefits of Lysolecithin in the intestine to obtain similar results to those found in non-ruminants, such improving overall gut health (Brautigan et al., 2017).

The propionate tended to increase by level of CSPOL, 280 and 400. In previous experiments, increments of up to 3 percentage-units in the digestibility of the NDF of the diet were reported with supplementation with sources of C16 (Onetti and Grummer, 2004; de Souza et al., 2017; de Souza and Lock, 2019), and this increase may occur due to two mechanism or the increase in the total number of bacteria (C16 incorporation in the membrane bacteria; (Vlaeminck et al., 2006)) or the lower passage rate (Piantoni et al., 2013), causing in both cases greater

digestion the feed particles, a consequently greater molar proportions of the volatile fatty acids, among them the propionate. The increase in propionate concentration, may be advantageous, propionate is the main precursor of glucose (Lemosquet et al., 2009).

The level of CSPOL, tended to cause a linear decrease on the intakes of DM and OM and these results agree with previous studies feeding calcium salts of palm oil (Harvatine and Allen, 2006b; Rabiee et al., 2012; Weld and Armentano, 2017). In a previous study from our Lab, feeding levels of CSPO, (negative control, 200g, 400g, and 600g as-fed), had a negative linear effect on DMI (16.9, 15.7, 15.9, 15.6 kg; P = 0.02) and OMI (15.2, 14.1, 14.3, 14.0 kg; P = 0.01), (Santos Neto, 2020). The negative effect of CSPO on DMI was extensively discussed by Allen (2000) and is associated with the hypophagic effect of FA present in CSPO (~38% in the present study). The reduction in DMI may also be associated with the alterations of ruminal microbial populations by supplemental FA (Hristov et al., 2005). We also observed a tendency for linear decrease on the intakes of DE and ME, probably associated with the decrease in DMI.

Additionally, we observed that levels of CSPOL had no effect on the intake of NEL. Similarly, a recent research has indicated that feeding levels of CSPO did no increase the intake of NEL on 200g, 400g and 600g, with intakes of 27.6, 28.1 and 28.3 Mcal/d of NEL respectively (Santos Neto, 2020). These results occur due to the decreased on DMI with the increase in the CSPO fed (Rabiee et al., 2012; Weld and Armentano, 2017).

It was observed that as cows advance in DIM, there is an increase in energy outputs to body reserves and energy balance, and a decrease on energy efficiency to NEL production/DE. Milk yield drives the energy requirements in dairy cows. In the peak period, there is a greater supply of nutrients to the mammary gland (Ferland et al., 2018), and in the post-peak there is a decrease in production and an increase in the energy available for gain (Hutjens, 2016). This was as observed in our study.

Increasing CSPOL linearly decreased MUN. In contrast, in a previous study by de Souza et al. (2017), it was reported no effects of CSPO on MUN. Similarly, Santos Neto (2020), did not report any effect of CSPO levels on MUN concentration. On the other hand, feeding Lysolecithin (CSPOL-400 vs CSPO) tended to increase MUN. Lee et al. (2019) evaluated the effects of two levels of supplemental Lysolecithin on rumen microbes, and they reported changes in microbial populations, with a decrease in *Rikenella* and *Paludibacter, Treponema bryantii* and *Bifidobacterium ruminantium* with increasing level of Lysolecithin, however lysolecithin did not significantly altered ruminal fermentation. In the present study, Lysolecithin did not alter protein intake and digestibility and there is no explanation for its effect on MUN.

In addition, milk fat content decreased linearly in CSPOL increased. This effect of CSPO on milk fat content is not in conformity with reports the previous studies. The milk fat content was not changed with supplementation with CSPO compared to the control treatment (de Souza et al., 2017) and it did not reduce when early dairy cows were be supplemented with increasing levels of CSPO (Santos Neto, 2020). Rabiee et al. (2012) conducted a meta-analysis and reported a positive effect of CSPO on milk fat concentration. This result in milk fat content can be associated with an increase in yield of C16:0 and C16:1 *cis*-9 (Batistel et al., 2017). In contrast, other studies, reported a positive effect of CSPO decreased the milk fat (Harvatine and Allen, 2006c; Rico et al., 2014). Calcium salts were designed as a inert fat to prevent constrains in rumen fermentation and digestion problems (Palmquist, 1991; Palmquist and Jenkins, 2017). The extent of the protection from the action of rumen microbes can be affected by ruminal pH, FA chain length, and the degree of unsaturation (Wu et al., 1991; de Souza et al., 2017). This can contribute to the formation of BH pathway intermediaries, such as *trans*-10 C18:1 FA and *trans*-10, *cis*-12 CLA, which are associated with MFD (Palmquist and Jenkins, 2017).

No treatment differences were observed by feeding levels of CSPOL on BW, BW changes and BCS. The mechanism by which supplemental fat did not affect the BW, BCS, BW, and BCS changes can be explained due to isomers of MFD. Lipogenic genes in mammary tissue were downregulated, whereas expression of lipogenic enzymes in adipose tissue underwent a significant increase (Harvatine et al., 2009), therefore it may have similarly affected the levels of CSPOL in the current study. Moreover, in our study, we observed a positive energy balance at both CSPO levels, in this case, the FA may be incorporated into triacylglycerol (Contreras et al., 2017), fortify this previous hypothesis.

5. CONCLUSIONS

Under the conditions of our study, Lysolecithin had no effect on nutrient intake and total-tract digestibility, metabolic-hormonal profile, energy partitioning, milk yield and on most milk component contents, BW and BCS of early lactating cows fed 400g of CSPO. In addition, feeding 280 to 400g of CSPO associated with Lysolecithin did not increase dairy cow performance. Whether the similar milk production of cows fed 280g or 400 of CSPO indicates a positive effect of Lysolecithin on fat utilization by lactating cows deserves further investigation.

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Table 1. Ingredient and nutritional composition of experimental concentrates and corn silage

		CSPO	CSPOL		
Item	Silage	400	280	340	400
Ingredient, % DM					
Ground corn		49.4	50.3	49.8	49.4
Soybean meal		40.0	40.5	40.3	40.0
Urea		0.95	0.97	0.96	0.95
Fat acid supplement[2]		4.86	3.40	4.13	4.86
Mineral and vitamin mix[3]		4.75	4.83	4.79	4.75
Nutrient composition, % DM					
OM	93.3	89.3	89.4	89.4	89.3
CP	7.93	25.7	26.1	25.9	25.7
NDF	47.1	10.2	10.4	10.3	10.2
ADF	25.4	3.34	3.39	3.37	3.34
Lignin	3.20	0.12	0.13	0.12	0.12
Ether-extract	3.28	6.48	5.32	5.90	6.48
Ash	5.12	10.6	10.6	10.6	10.6

[1] CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[2] Fat supplements were (1) 400g CSPO; EnerFat®, Kemin Industries, Inc., 85.2% FA (40.4% C16:0; 3.9% C18:0; 31.8% C18:1 cis-9; 6.5% C18:2 cis-9, cis-12; and others <4% each); and levels of CSPO whith 1% of Lysoforte eXtend® Kemin Industries, Inc. (98% of Lysolecithin), (2)280g, (3)340g, (4)400g of CSPOL.

[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.

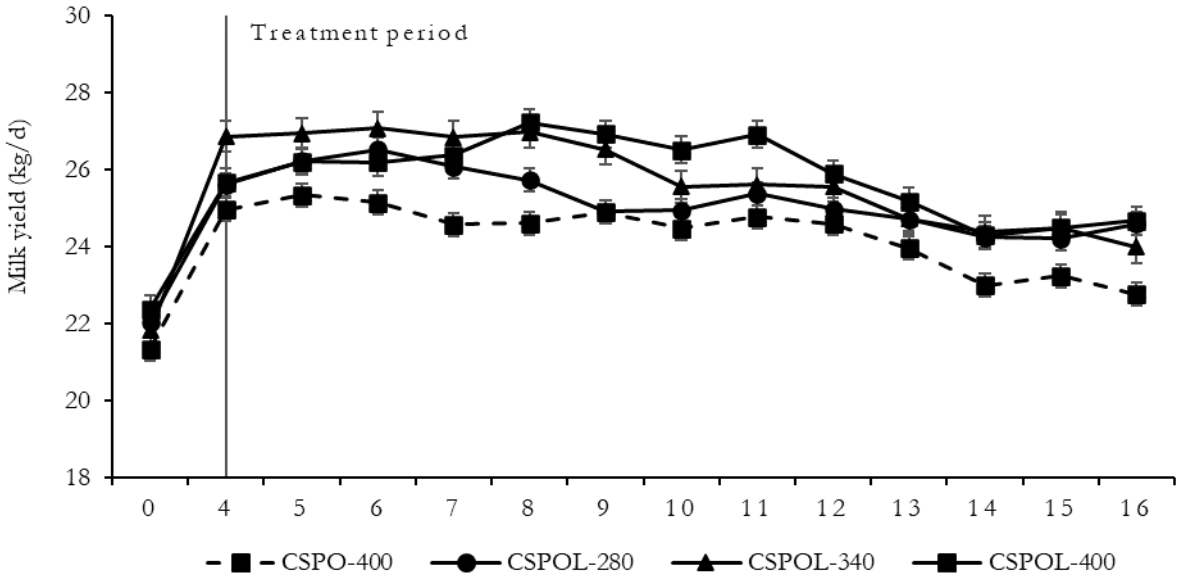

Figure 1. Effects of dietary treatments on milk yield over time during the treatment period (from 4 to 16 week). Treatments were a control (concentrate with 400g of CSPO without Lysolecithin (CSPO); and concentrate with three levels of CSPO with 1% Lysolecithin (CSPOL), 280g, 340g and 400g. The milk yield was not affected ($P > 0.05$) by treatment. The black bar presents the start of the treatment period. Error bars represent SEM.

Table 2. Effects of supplementation with calcium salts of palm oil (CSPO) or calcium salts of palm oil with 1% Lysolecithin (CSPOL) on lactation performance of dairy cows (n=44).

	Treatment (Trt)[1]					P-value[2]					
	CSPO	CSPOL									
Item	400	280	340	400	SEM	Lyso	L	Q	Trt	week	Trt x week
Yield (kg/d)											
Milk	24.3	25.2	25.8	25.6	1.12	0.20	0.72	0.84	0.43	<0.01	0.26
Fat	0.92	0.97	0.89	0.92	0.04	0.95	0.30	0.11	0.36	0.05	0.95
Protein	0.75	0.77	0.78	0.76	0.03	0.82	0.78	0.95	0.71	0.02	0.81
Casein	0.57	0.60	0.61	0.58	0.06	0.14	0.47	0.57	0.51	<0.01	0.93
Lactose	1.12	1.13	1.17	1.17	0.05	0.36	0.47	0.76	0.71	<0.01	0.59
3.5% FCM[3]	25.6	26.3	26.4	25.7	1.28	0.97	0.57	0.72	0.86	0.02	0.93
ECM[4]	25.4	26.1	26.3	25.5	1.23	0.90	0.63	0.78	0.85	0.02	0.94
Cumulative milk yield (kg)	2661	2842	2843	2812	148	0.36	0.85	0.52	0.64	NA[5]	NA
Milk composition (%)											
Fat	3.82	3.94	3.67	3.54	0.12	0.06	0.01	0.13	0.06	0.22	0.72
Protein	3.08	3.10	3.09	3.04	0.05	0.53	0.37	0.82	0.80	0.07	0.86
Casein	2.42	2.36	2.37	2.29	0.03	0.88	0.41	0.71	0.51	0.07	0.56
Lactose	4.61	4.60	4.60	4.63	0.06	0.64	0.58	0.95	0.94	0.02	0.87
MUN (mg/dL)	15.3	17.9	16.1	16.6	0.58	0.07	0.05	0.15	0.08	0.25	0.79
BW (kg)	525	515	510	505	23.0	0.36	0.65	0.88	0.81	<0.01	0.98
BW change (kg/d)	0.25	0.09	0.12	0.24	0.12	0.94	0.32	0.42	0.63	NA	NA
BCS	2.74	2.87	2.84	2.75	0.11	0.13	0.40	0.50	0.18	<0.01	0.40

[1] CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[2] Probability associated with the effects of treatment, time and treatment × time interaction, Lyso = contrast CSPOL-400 versus CSPO-400; L= linear effect of CSPOL; Q =quadratic effect of CSPOL.

[3]3.5% FCM = [(0.4324 × milk yield) + (16.216 × kg of milk fat)].

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

[5]Not applicable.

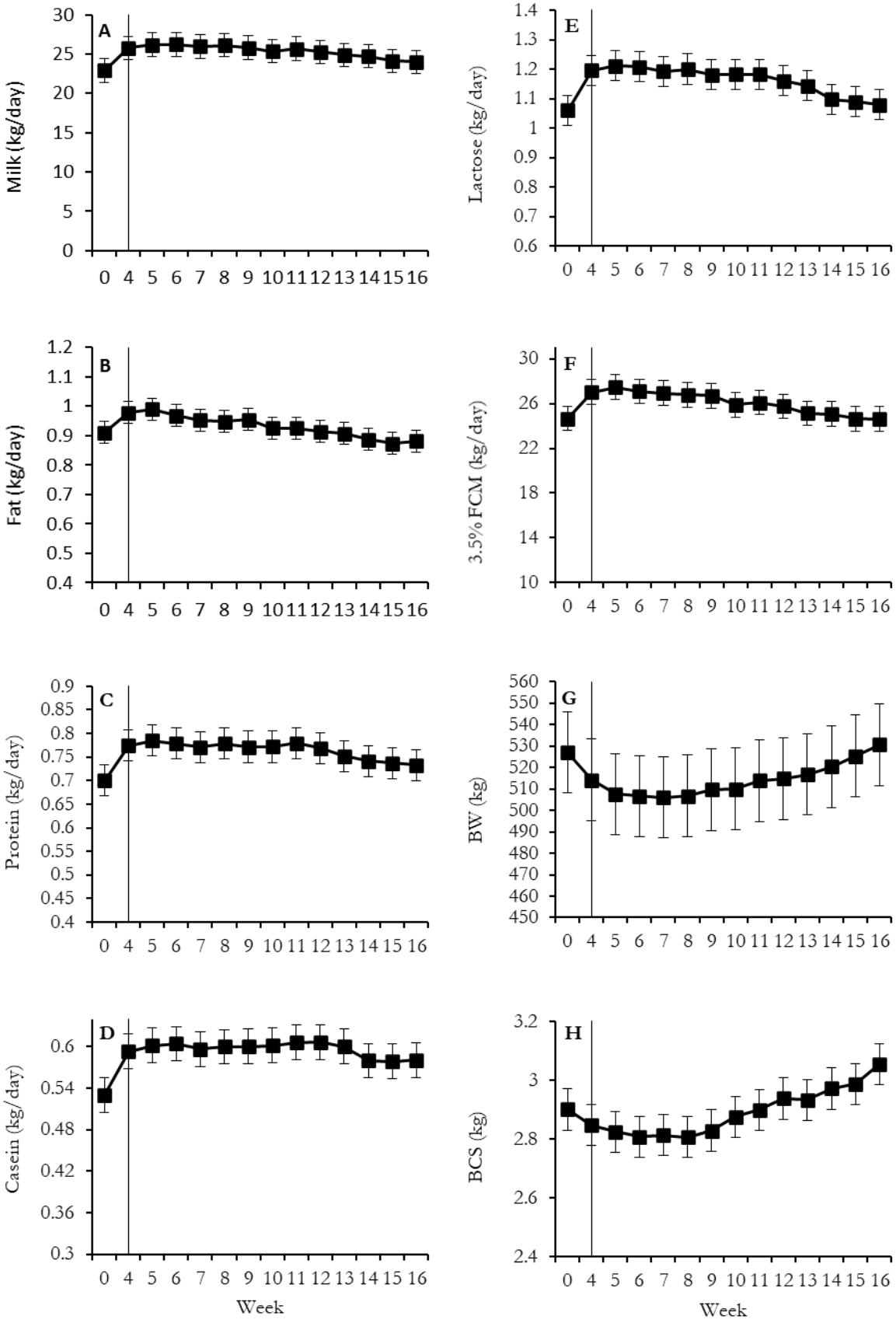

Figure 2. Effects of time about milk yield (A), fat yield (B), protein yield (C), casein yield (D), lactose yield (E), 3.5% FCM yield (F), body weight (G), and body condition score (H). The black bar presents the start of the treatment period. Error bars represent SEM.

Table 3. Effects of supplementation with calcium salts of palm oil (CSPO) or calcium salts of palm oil with 1% Lysolecithin (CSPOL) on nutrients intake, and apparent total tract digestibility during two periods 35 and 65 DIM, of dairy cows during treatment period (n=44).

	Treatment (Trt)[1]										
	CSPO	CSPOL				P-value[2]					
Item	400	280	340	400	SEM	Lyso	L	Q	Trt	Period	Trt x Period
Intake (kg/d)											
DM	17.2	17.6	17.2	16.9	0.27	0.41	0.07	0.23	0.34	0.48	0.62
OM	15.7	16.0	15.7	15.5	0.25	0.42	0.08	0.23	0.34	0.32	0.63
CP	2.75	2.81	2.77	2.74	0.05	0.66	0.13	0.28	0.49	0.15	0.91
NDF	5.22	5.37	5.18	5.08	0.16	0.48	0.12	0.27	0.47	0.13	0.74
FA	0.64	0.56	0.60	0.64	0.01	0.67	<0.01	0.15	<0.01	0.87	0.92
Digestibility (%)											
DM	69.8	69.1	69.0	69.8	0.82	0.89	0.40	0.70	0.71	0.34	0.95
OM	71.5	70.9	70.9	71.7	0.76	0.87	0.41	0.72	0.74	0.11	0.93
CP	74.4	73.8	74.3	75.3	0.92	0.43	0.19	0.60	0.61	0.28	0.96
NDF	46.4	46.0	46.6	46.7	1.93	0.84	0.96	0.79	0.87	0.41	0.96
FA	81.8	79.5	81.9	83.6	2.05	0.19	<0.01	0.10	0.04	0.11	0.24
Feed efficiency[3]	1.50	1.54	1.60	1.55	0.08	0.52	0.92	0.94	0.67	0.25	0.98

[1] CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[2] Probability associated with the effects of treatment, time, and treatment × time interaction, Lyso = contrast CSPOL-400 versus CSPO-400; L= linear effect of CSPOL; Q =quadratic effect of CSPOL.

[3]Feed efficiency = ECM/DMI.

Table 4. Effects of supplementation with calcium salts of palm oil (CSPO) or calcium salts of palm oil with 1% Lysolecithin (CSPOL) on energy balance and energy partitioning at 35 and 65 DIM, of dairy cows during treatment period (n=44).

	Treatment (Trt)[1]										
	CSPO	CSPOL				P-value[2]					
Item	400	280	340	400	SEM	Lyso	L	Q	Trt	Period	Trt x Period
Energy intake[3], Mcal/d											
DE	60.4	60.7	59.6	59.3	1.06	0.40	0.29	0.54	0.68	0.19	0.65
ME	53.3	53.0	52.3	52.4	0.94	0.43	0.58	0.83	0.78	0.14	0.64
NEL	34.2	33.9	33.5	33.6	0.62	0.44	0.71	0.94	0.790	0.13	0.64
Energy output, Mcal/d											
Milk[4]	17.5	18.4	18.7	17.9	0.88	0.64	0.59	0.69	0.56	0.21	0.91
Maintenance[5]	8.73	8.69	8.57	8.51	0.30	0.43	0.51	0.85	0.84	0.74	0.40
Body reserves[6]	0.90	0.82	0.63	0.70	0.16	0.36	0.57	0.76	0.60	<0.01	0.62
Energy balance[7], Mcal/d	7.12	5.99	5.61	6.48	1.34	0.62	0.70	0.73	0.66	0.01	0.90
Efficiency											
NEL milk/DE	0.29	0.30	0.32	0.30	0.02	0.47	0.92	0.93	0.51	0.14	0.96
NEL production[8]/DE	0.45	0.46	0.47	0.46	0.02	0.69	0.88	0.92	0.82	0.02	0.95

[1] CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[2] Probability associated with the effects of treatment, time, and treatment × time interaction, Lyso = contrast CSPOL-400 versus CSPO-400; L= linear effect of CSPOL; Q =quadratic effect of CSPOL.

[3] Diet energy values were calculated based on nutrient digestibility (Boerman et al., 2015b) using equations (NRC, 2001) and multiplied by DMI to estimate energy intake (Mcal/d).

[4] NEL milk (Mcal/d) = Milk yield (kg) × (0.0929 × fat % + 0.0563 × true protein % + 0.0395 × lactose %), (NRC, 2001).

[5] NEL maintenance (Mcal/d) = 0.08 Mcal/kg × BW (kg)$^{0.75}$ (NRC, 2001).

[6] Body reserves output (Mcal/d) = [(2.88 + 1.036 x BCS) x ΔBW], where BCS was the average BCS for study and ΔBW was BW change (NRC, 2001).

[7] Energy balance (Mcal/d) = NEL intake (Mcal/d) − milk NEL (Mcal/d) − NEL maintenance requirement (Mcal/d).

[8] NEL production = milk NEL + NEL required for maintenance.

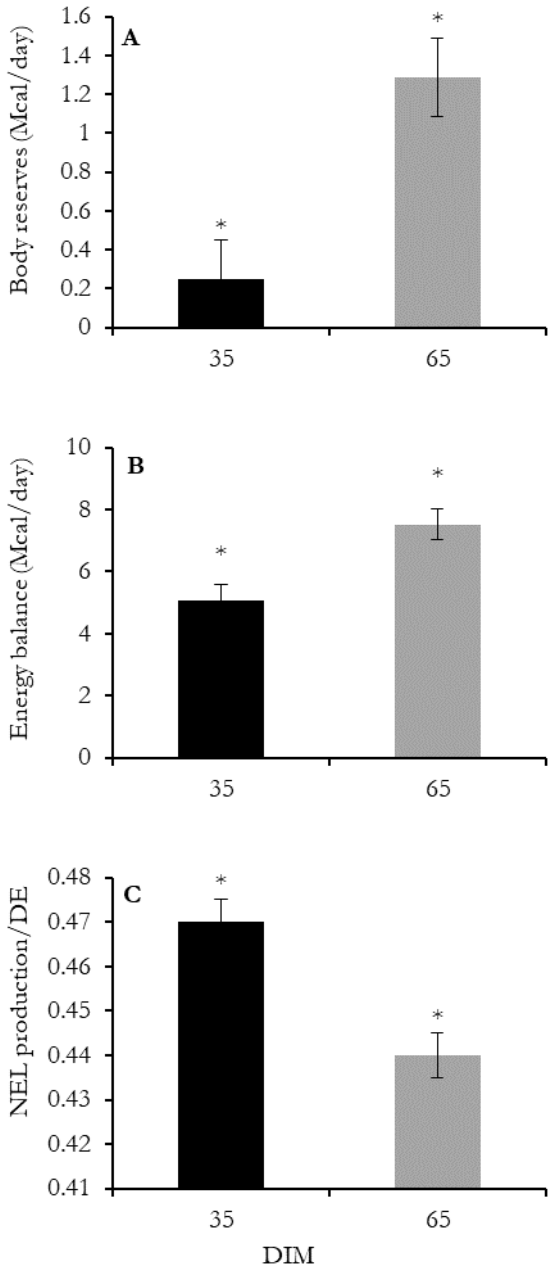

Figure 3. Effects of time on energy outputs in body reserves (A), energy balance (B), and NEL production/DE. Significances at * $P \leq 0.05$. Error bars represent SEM.

Table 5. Blood metabolite concentrations[1] for cows fed treatment diets (n=44).

	Treatment (Trt)[1]					P-value[2]					
	CSPO	CSPOL									
Item	400	280	340	400	SEM	Lyso	L	Q	Trt	Period	Trt x Period
Protein, g/dL	8.92	8.94	8.63	8.84	0.27	0.83	0.79	0.61	0.83	0.01	0.30
Glucose, mg/dL	57.7	57.6	59.7	61.4	2.38	0.03	0.05	0.97	0.04	0.15	0.61
Insulin, ng/L	0.80	0.73	0.66	0.60	0.36	0.58	0.51	0.85	0.89	0.05	0.13
NEFA[4], mmol/L	0.30	0.31	0.32	0.32	0.03	0.67	0.92	0.95	0.94	0.29	0.86
Urea, mg/dL	46.5	48.1	45.1	43.5	3.88	0.39	0.21	0.47	0.62	0.42	0.51

[1] Blood samples collected at 35 and 65 DIM of treatment period.

[2]CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[3]Probability associated with the effects of treatment, time, and treatment × time interaction, Lyso = contrast CSPOL-400 versus CSPO-400; L= linear effect of CSPOL; Q =quadratic effect of CSPOL.

[4]NEFA = no esterified fatty acids.

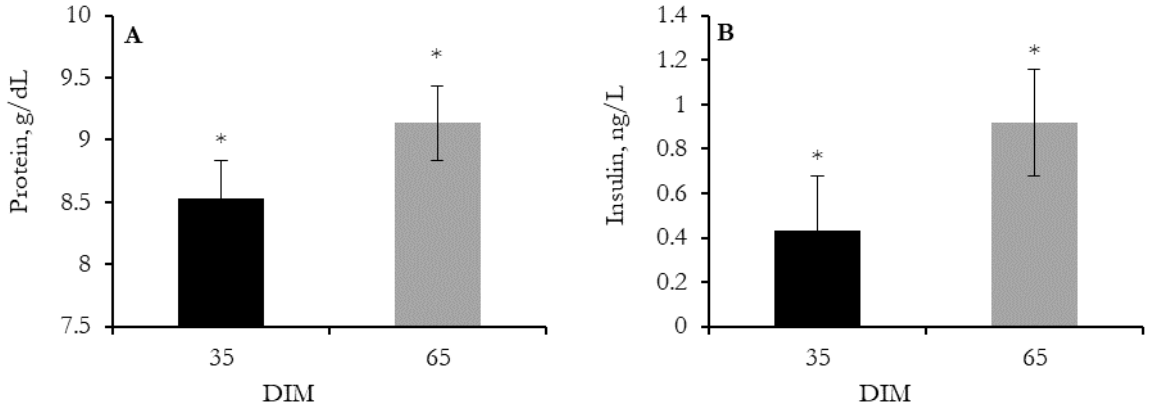

Figure 4. Effects of time on concentrations of blood protein (A), and insulin (B). Significances at * $P \leq 0.05$. Error bars represent SEM.

Table 6. Effects of supplementation with calcium salts of palm oil (CSPO) or calcium salts of palm oil with 1% Lysolecithin (CSPOL) on ruminal fermentation, nutrients intake, and apparent total tract digestibility in ruminally cannulated dairy cows (n=3).

Item[3]	Treatment (Trt)[1]				P-value[2]		
	CSPO	CSPOL					
	400	280	400	SEM	Lyso	D	Trt
Intake (kg/d)							
DM	16.1	16.7	16.1	0.46	0.98	0.32	0.50
OM	15.0	15.5	15.0	0.44	0.93	0.32	0.50
CP	2.45	2.50	2.45	0.17	0.92	0.34	0.49
NDF	4.38	4.62	4.39	0.15	0.98	0.33	0.51
FA	0.56	0.54	0.56	0.03	0.92	0.87	0.96
Digestibility (%)							
DM	63.8	63.2	64.8	2.61	0.77	0.63	0.73
OM	65.6	65.2	66.9	2.44	0.64	0.35	0.64
CP	67.9	67.5	68.3	3.26	0.26	0.41	0.47
NDF	41.1	39.5	41.5	1.17	0.44	0.53	0.39
FA	78.9	75.9	78.4	3.77	0.43	0.78	0.69
Ruminal parameters							
pH							
Mean	6.50	6.59	6.62	0.13	0.31	0.81	0.53
Minimum	6.26	6.23	6.21	0.12	0.63	0.81	0.88
Maximum	6.98	7.10	7.04	0.10	0.59	0.59	0.56
Total VFA, mM	70.6	79.2	72.9	4.69	0.52	0.14	0.15
% of total VFA							
Acetate (A)	63.6	62.7	61.2	1.25	0.15	0.33	0.31
Propionate (P)	19.1	20.9	22.6	1.77	0.09	0.33	0.10
Isobutyrate	1.18	0.94	1.03	0.08	0.14	0.38	0.11
Butyrate	12.8	12.1	11.7	0.57	0.20	0.60	0.39
Isovalerate	2.02	2.06	2.06	0.09	0.79	0.96	0.94
Valerate	1.21	1.19	1.34	0.05	0.05	0.03	0.06
A: P, ratio	2.98	3.10	3.19	0.24	0.45	0.73	0.74
NH_3-N mg/dL	7.27	13.4	11.3	2.01	0.19	0.52	0.16

[1] CSPO = calcium salts of palm oil; CSPOL = calcium salts of palm oil with 1% Lysolecithin.

[2] Probability associated with the effects of treatment, Lyso = contrast CSPOL-400 versus CSPO-400; D = effect of dose CSPOL.

[3] VFA = Volatile Fat Acid; NH_3-N = Ammonia Nitrogen.