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Conjugated linoleic acid supplementation during the transition period increased milk production in primiparous and multiparous dairy cows

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ABSTRACT

Trans-10, *cis*-12 conjugated linoleic acid (CLA), a bioactive fatty acid, has the potential to alter energy metabolism in lactating dairy cows by marginally reducing milk fat synthesis in the mammary gland. The objective of this study was to determine the effects of pre- and postpartum CLA supplementation on lactation performance in a commercial dairy setting. Multiparous (mp) and primiparous (pp) Holstein cows managed in an automatic milking system were blocked by expected calving month, and randomly assigned to either a CLA supplementation (CLA-ME; mp n = 99, pp n = 38) or control (CTL; mp n = 97, pp n = 38) group. Cows were supplemented 100 g/d of lipid encapsulated CLA methyl esters containing 10 g each of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, mixed 50:50 with soybean meal, and delivered via an automated mineral dispenser. Supplementation continued from −21 d pre-calving through 30 d in milk (DIM) for mp or 70 DIM for pp. Daily milk yield, fat and protein content, body weight, and rumination minutes were recorded and averaged by wk for the first 100 DIM. Milk yield was increased ($P = 0.04$) during the supplementation period (44.7 vs. 46.6 ± 0.64 kg/d) for mp CLA-ME cows and was increased ($P = 0.03$) during the post-supplementation period (31.3 vs. 34.2 ± 0.91 kg/d) for pp CLA-ME cows. Milk fat content was decreased ($P = 0.03$) during the supplementation period for mp CLA-ME cows and post-supplementation period for pp CLA-ME cows ($P = 0.02$); but only tended to be decreased ($P = 0.09$) in the supplementation for pp CLA-ME cows. Fat yield was not altered ($P > 0.10$) in either period for mp and pp CLA-ME. Supplementation with CLA did not change milk protein content or yield during the supplementation period for either mp or pp CLA-ME. During the post-supplementation period protein content was decreased ($P = 0.02$); however, yield remained unchanged for mp CLA-ME cows while protein content was unchanged and yield increased for pp CLA-ME cows. Body weight and rumination minutes were unchanged by CLA supplementation. Intervals to first estrus, first service, and conception were similar for CTL and CLA-ME mp and pp cows. First service conception rate was similar for mp CTL and CLA-ME cows; but, tended to be increased ($P = 0.10$) in pp CLA-ME compared to CTL.

Abbreviations: ACC, acetyl-CoA carboxylase; AMS, automatic milking system; CLA-ME, conjugated linoleic acid supplement group; CTL, control group; FASN, fatty acid synthase; HR, hazard's ratio; ICAR, International Committee for Animal Recording; PMR, partial mix ration; SREBP, sterol regulatory element binding protein; T4C, Time for Cows.

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These results suggest that transition period CLA supplementation altered energy metabolism to increase milk production during the supplementation period for mp cows and post-supplementation period for pp cows without adversely affecting reproductive performance in mp cows and improving conception in pp cows.

1. Introduction

The transient period of stress and adaptation during the onset of lactation poses a physiological and metabolic challenge for dairy cows as the exponential increase in milk production is only partially met by the delayed increase in feed intake (Bauman and Currie, 1980). This period of negative energy balance (**NEB**), and any failure to fully adapt manifests itself as an increase in the risk of metabolic disorders (Drackley, 1999), impaired performance (Goff and Horst, 1997), and depressed reproductive efficiency (Beam and Butler, 1999; Webb et al., 1999; Butler, 2001). Nutritional strategies to reduce milk energy output during heat stress or weather-related feed shortages have been employed (Bauman et al., 2011). Modulating or reducing milk energy output during the transition period to mitigate NEB might prove beneficial.

Conjugated linoleic acid (**CLA**) is a family of geometrical and positional isomers of linoleic acid produced as intermediates during rumen biohydrogenation. It is well documented that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis (Baumgard et al., 2000, 2001, 2002; Peterson et al., 2002) and reduces milk fat yield in a dose-dependent manner (Peterson et al., 2002) that is recovered after cessation of CLA supplementation (Baumgard et al., 2000). *trans*-10, *cis*-12 CLA depresses milk fat via inhibition of *de novo* fatty acid synthesis and uptake of preformed fatty acids (**FA**) in the mammary gland by down-regulating gene expression of key lipogenic enzymes (Bauman et al., 2011; Hussein et al., 2013). Milk fat is the most energetically expensive component of milk, representing 50% of milk energy and can account for up to 35% of net energy intake in early lactation (Bauman and Davis, 1974; Bauman and Currie, 1980; Kay et al., 2006). A decrease in milk fat excretion could spare energy for other uses such as milk production, production of other milk components, or body growth.

In cases of early lactation CLA supplementation, reduction of milk fat content was accompanied by increased milk yield (Bernal-Santos et al., 2003; Moallem et al., 2010; Hutchinson et al., 2012; Schlegel et al., 2012). We hypothesized that CLA supplementation would alter energy metabolism to increase milk production during the transition to lactation period. The objective of the present work was to examine the effect of supplementing rumen-protected CLA pre- and postpartum on milk and component yields in a commercial dairy setting.

2. Materials and methods

2.1. Animals and treatments

This study was conducted on a commercial dairy in central Wisconsin between November 2013 and February 2015. Animal use and handling protocols were approved by the University of Wisconsin-Madison Animal Care and Use Committee. A total of 350 multiparous (n = 265) and primiparous (n = 85) animals were separated by parity (1 and ≥2), blocked by expected calving month within parity group, and randomly assigned to either a control group that received no supplement (**CTL**) or a group that received CLA supplement (**CLA-ME**). In addition to the same mixed rations, cows within the CLA-ME treatment group received an additional 100 g/d of CLA methyl ester containing 10 g each of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA (Lutrell® Pure; BASF, Ludwigshafen, Germany) in a lipid-encapsulated form to remain insoluble and resistant to ruminal biohydrogenation processes. The product was mixed 50:50 with soybean meal and a total of 200 g of the mixture was supplemented to cows within the CLA-ME treatment group. Animals were supplemented from −21 days relative to calving until 30 days in milk (DIM) for multiparous animals and 70 DIM for primiparous animals.

2.2. Management and supplementation

Animals were housed in 7 pens of equal size, and animals within each pen had access to one Lely A4 Astronaut (Lely Industries, Maassluis, The Netherlands) automatic milking system (AMS). Animals remained in their assigned pen during the current lactation cycle and had access 24 h/d to only the AMS adjoined to their pen in a free cow traffic system. Individual animals were identified by their transponders as they entered the robot and were either milked or forced to exit the robot based on time since their previous milking, with a minimum visit cycle set at 4 h. Milking occurred on a voluntary basis, but if an individual cow had not visited the AMS for >12 h, farm staff directed her to be milked.

During lactation, animals received a partial mixed ration (PMR) that was delivered once daily in the morning and offered ad libitum. The remainder of the total balanced ration was supplemented in the AMS as a pelleted feed during milkings. The PMR fed during the trial consisted of 530 g/kg forage and 370 g/kg concentrate (DM basis). Table 1 summarizes the composition of dry cow, pre-fresh, and post-fresh diets fed to the herd during the trial. During the dry period, all animals were fed and housed in the same freestall barn. During the pre-fresh period, both parity groups were housed in the same pen that was separated by gates to equally split the alley, stalls, and bunk space into two pens: a CTL and a CLA-ME pen.

Table 1
Summary of feed ingredients for target diets fed during the trial[a].

	Diet		
Ingredient, g/kg DM	Dry Cow	Prefresh	Postfresh
AMS[b] concentrate, pelleted			149
Alfalfa haylage			64
Corn silage	306	590	211
Stalklage[c]	268		
Baleage			114
Dry hay	300		71
Grain mix	120		
Wheat straw		200	
Dry cow mineral supplement	6		
Prefresh concentrate mix[d]		210	
Postfresh concentrate mix[e]			390

[a] Values represent an example diet that was the targeted diet composition.
[b] Automatic milking system.
[c] Stalklage was included in the postfresh diet to balance NDF when feed inventory was limited.
[d] Concentrate mix targeted for (g/100 g): 65.2 g Bio-Chlor™, 6.2 g calcium sulfate, 6.2 g magnesium sulfate, 3.7 g ReaShure®, 3.7 g OmniGen-AF®, 3.2 g blood meal, 0.9 g salt, 0.9 g yeast powder, 0.072 g chromium propionate, 0.32 g magnesium oxide, 3.4 g biotin, 0.2 g trace mineral supplement (4.9 mg/kg cobalt, 64.5 mg/kg copper, 5.1 mg/kg iodine, 323 mg/kg iron, 279 mg/kg manganese, 310 mg/kg zinc), 0.078 g selenium, 0.3 g rumensin, 18,545 IU/kg Vitamin A, 1636 IU/kg Vitamin D, and 93 IU/kg Vitamin E.
[e] Concentrate mix targeted for (g/100 g): 3.7 g OmniGen-AF®, 30.4 g blood meal, 30.3 g sodium bicarbonate, 13.3 g salt, 1.8 g yeast powder, 4.59 g magnesium oxide, 1.1 g biotin, 1 g trace mineral supplement (21 mg/kg cobalt, 243 mg/kg copper, 18.5 mg/kg iodine, 789 mg/kg iron, 945 mg/kg manganese, 1098 mg/kg zinc), 0.3 g selenium, 0.7 g rumensin, 37,273 IU/kg Vitamin A, 5682 IU/kg Vitamin D, and 125 IU/kg Vitamin E.

Table 2
Chemical composition of diets fed during the trial[a].

	Diet[b]					
	Dry Cow		Prefresh		Lactating	
Chemical composition, g/kg DM	Mean	SE	Mean	SE	Mean	SE
DM	368	6.5	465	12.9	482	10.9
CP	143	3.6	144	6.4	154	2.5
ADF	329	0.8	282	11.8	244	8.3
aNDF	443	7.0	397	10.9	326	11.9
aNDFom	409		378	9.7	309	11.7
Lignin	49.5	1.9	33.9	1.5	31.3	1.6
Starch	127	27.2	165	21.9	224	16.4
Ether extract	37.6	8.4	41.1	1.3	39.4	2.5
Ash	87.4	2.3	87.0	4.9	85.6	5.5
NFC[c]	312	16.2	363	14.3	425	11.3
NE_L[d], MJ/kg DM	6.0	0.2	6.5	0.1	6.7	0.1

[a] Values represent the mean and standard error of samples collected and analyzed throughout the trial.
[b] Composition represents TMR of dry cow and prefresh diet, and PMR of lactating diet.
[c] Calculated as 100–(CP g/100 g + NDF g/100 g + ether extract g/100 g + ash g/100 g; NRC, 2001).
[d] Calculated according to NRC (2001).

All pre-fresh animals received the same TMR once daily for ad libitum intake. The CLA-ME group was supplemented by top-dressing the CLA and mixing into the TMR immediately after feeding. No performance data were collected during the pre-fresh period.

Feed samples from the pre-fresh TMR and post-fresh PMR were collected routinely while cows were supplemented with CLA and dried at 105 °C for 24 h in a forced-air oven to determine DM content and ground to pass a 1-mm screen using a Wiley mill (model #4, Thomas Scientific, Swedesboro, NJ). Samples were analyzed (Dairyland Laboratories Inc., Arcadia, WI) for CP (method 990.03, AOAC, 1996), ADF (method 973.18, AOAC, 1996), NDF using alpha amylase and sodium sulfite (method 2002.04, AOAC, 2005), lignin using sulfuric acid (method 973.18, AOAC, 1996), starch (2014.10, Hall, 2015), and ether extract (method 920.39, AOAC, 1996). Feed sample NFC and NE_L were calculated based on the NRC (2001). A summary of the chemical composition of diets are displayed in Table 2.

After calving, multiparous animals were moved to the AMS pen they were in during their previous lactation and primiparous animals were assigned to an AMS pen in a rotating basis to maintain stocking density and parity balance. While being milked in the AMS, animals were automatically supplemented a portion of their daily-allocated concentrate pellet: between 1 and 8 kg/d based on their parity, DIM, and milk yield. A summary of daily AMS pellet allocation is shown in Table 3. If animals were not offered their entire daily AMS pellet allocation, 50% of the remainder was added to the available amount the following day. The AMS pellet was formulated to meet a target NE_L of 1.8 Mcal/kg, DM. Throughout the trial the pellet

Table 3
Feed table for AMS[a] pellet allocation during the first 100 DIM..

	DIM	Milk yield, kg/d	AMS pellet, kg/d
Multiparous	<10		3.0
	<21		4.3
	21		6.8
	>22	<27	3.2
		<36	4.1
		<45	5.0
		<59	6.4
		<72	7.7
Primiparous	<10		2.3
	<30		3.2
	30		5.5
	>31	<27	3.6
		<36	4.1
		<45	5.0
		<59	6.4
		<72	7.7

[a] Automatic milking system.

consisted of corn gluten feed, wheat middlings, soy hulls, soybean meal, calcium carbonate, salt, sweeteners, and flavorings such as anise.

During the lactation period CLA-ME animals were supplemented in the AMS during each milking. Upon entering the AMS, the supplement was automatically dispensed to individual animals using a PowerDos feeding system (Hanskamp AgroTech BV, Zelhem, The Netherlands) housed in the AMS. The PowerDos system delivered the supplement from a hopper via a pneumatic stainless steel dosage mechanism. The mechanism delivered 18 g of CLA supplement per dose and was programmed to deliver a number of doses at each animal's AMS milking based on hours since the previous milking according to the usual practice of linear accumulation of the feed allowance. The CLA-ME animals received a total of 11 doses in a 24 h period to achieve the target dose of 200 g/d of the CLA supplement. The feeding system was calibrated before the beginning of the trial, monitored weekly, and recalibrated when necessary. A sample of the CLA and soybean supplement was analyzed for FA by gas-liquid chromatography. The FA were extracted and methylated by the method of (Sukhija and Palmquist, 1988) and FA methyl esters analyzed on a Perkin Elmer Clarus 500 TotalChrom Data Handling System (Norwalk, CT) and separated using an open tubular fused silica capillary column [100 m × 0.25 mm (i.d.)] coated with CP-Sil 88 (Chrompack #CP7489, Varian Inc., Walnut Creek, CA). Helium was the carrier gas at a flow rate of 0.90 mL/min. Initial column temperature was set at 50 °C and held for 0.4 min and increased to 190 °C at a rate of 4 °C/min and held for 95 min. Inlets and detector temperatures were set at 255 °C. Based on retention times determined using methyl ester standards (Matreya LLC., State College, PA), the content of the CLA isomers in the CLA and soybean meal supplement was 54.2 mg/g of *cis*-9, *trans*-11 and 51.8 mg/g of *trans*-10, *cis*-12 CLA. Based on a 200 g per day supplementation rate, the total amount of CLA isomers provided would have been 10.8 g of *cis*-9, *trans*-11 and 10.4 g of *trans*-10, *cis*-12 CLA.

The herd's breeding program was managed by farm staff and they recorded all events associated with reproduction. Following is a brief description of the herd's breeding program. All animals received a $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine; Lutalyse, Zoetis) injection between 28 and 34 DIM. Animals were artificially inseminated 12 h post-estrus after a 55 d voluntary waiting period. Activity monitors were used to aid in estrus detection and trained farm staff performed artificial insemination (AI). Animals that were not inseminated by 80 DIM were subjected to an ovsynch protocol [a GnRH (100 ug of gonadoreline diacetate tetrahydrate; Fertagyl, Merck Animal Health, De Soto, KS) injection was administered, followed by $PGF_{2\alpha}$ 7 d later, a second GnRH injection 56 h later, after which timed AI occurred 12–16 h later]. Pregnancy was diagnosed by ultrasonography performed by the herd veterinarian 32 ± 2 d after AI. Animals were enrolled only once during the study. Animals in the study that calved again during the duration of the study were not enrolled in the study a second time.

2.3. Data collection

Milk yield was measured at each AMS milking and recorded and stored by the Lely AMS proprietary software reporting system, Time for Cows (T4C; Lely, Maassluis, The Netherlands). Individual cow milk yield was measured in a collection jar fixed with a load cell that was calibrated every 6 months and ICAR (International Committee on Animal Recording) approved. Milking frequency (milkings per day) was recorded based on AMS visits that resulted in a milking. If a cow did not meet the milking interval or there was a connection problem upon entrance to the AMS, the cow was not supplied concentrate, forced to exit, and the visit was not considered a milking. Daily milk fat, true protein, and lactose content were measured by the AMS in-line measurement system (MQC2: Milk Quality Control, type 2; Lely, Maassluis, The Netherlands) by irradiating milk with 4 individual light-emitting diodes in the same visual spectrum as previously employed (Aernouts et al., 2011). Contralateral photo diodes measured light transmittance and transformation of the scattering effects of fat-globules and other particles in raw milk. Daily measures were calibrated against laboratory values of fat, true protein, and lactose (Berg and Vijverberg, 2002) obtained from monthly milk samples collected for each cow by an automatic milk-sampling

device (Lely Sampling Shuttle; Lely Industries, Maassluis, The Netherlands) mounted on the AMS unit and preserved with 2-bromo-2-nitropropane-1,3-diol until analysis. Monthly milk samples were analyzed for milk fat, true protein, and lactose by Fourier transform infrared spectrometry using a FOSS MilkoScan FT6000 (AgSource Laboratories, Menomonie, WI) and sample results were recorded in the T4C system for monthly calibration of the in-line measurement system. During a case of mastitis or mastitis attention alerted by T4C, that milking was not used for calibration. Values from the MQC2 for every milking, and the daily weighted average, were calculated and recorded by T4C for later export and statistical analysis. Yield of 3.5% fat-corrected milk (FCM) and the energy content of milk was calculated according to the NRC (2001).

Milk samples were collected from 21 cows (11 CTL, 10 CLA-ME) at 7 ± 1 DIM for analysis of vitamins A and E, and milk FA composition. Milk samples were taken at the first milking of the day after 8 h without access to the AMS by collecting a composite milk sample from the collection jar at the AMS after milking. Whole milk was aliquoted and stored at −20 °C until analysis for vitamins A and E. A whole milk aliquot (approximately 30 mL) was centrifuged at 17,800 × *g* for 30 min and the fat cake removed and stored at −20 °C until analysis of milk fatty acid composition. For vitamins, milk was saponified with potassium hydroxide and vitamins extracted using hexane and then re-solubilized in a 70% acetonitrile, 20% methylene chloride, and 10% methanol mobile phase solution that was analyzed by a Waters H-class Acquity UPLC system (Waters Corporation, Milford, MA) using reverse phase chromatography on a BEH C18 1.7 μm, 2.1 × 50 mm column (Waters Corporation, Milford, MA) with a flow rate of 0.5 mL/min. A photo diode array detector was used to quantify vitamins A and E and spectral purity at 325 nm and 292 nm respectively. Relative retention was used to confirm vitamin identity.

For FA, lipids were extracted with hexane:isopropanol and methyl esters prepared in sodium methoxide based on the method by Chouinard et al. (1999). Fatty acid methyl esters were quantified by gas chromatography using an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) and separated using a fused-silica capillary column [SP-2560, 100 m × 0.25 mm i.d. with 0.2-μm film thickness] (Supelco Inc., Bellefonte, PA). Hydrogen was the carrier gas at a flow rate of 1 mL/min. Initial column temperature was set at 80 °C, was increased by 2 °C/min to 190 °C, and held for 15 min and then increased 5 °C/min to 215 °C and held for 20 min. Airflow was 400 mL/min and hydrogen gas flowed at 25 mL/min. Inlet and detector temperatures were set at 250 °C with a 100:1 split ratio. Fatty acid peaks were identified using FAME standards (GLC 461, GLC 780, and pure *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA, NuChek Prep Inc., Elysian, MN; Bacterial Acid Methyl Ester Mix, 47080-U, Sigma-Aldrich Inc, St. Louis, MO; and GLC 110 mixture, Matreya LLC., State College, PA). Recovery of individual FA were determined using an equal weight reference standard (GLC 461; NuChek Prep Inc.), and correction factors for individual FA were carried out as described by Rico and Harvatine (2013). Under these conditions *cis*-9, *trans*-11 CLA is expected to elute with the isomers *trans*-7, *cis*-9 CLA and *trans*-8, *cis*-10 CLA; however, *cis*-9, *trans*-11 CLA represents the majority of the peak (Fritsche et al., 1999). Milk FA desaturase indexes were calculated as an estimation of the activity of the stearoyl CoA desaturase enzyme [product/(substrate + product)].

Body weight was measured at each AMS visit for all animals via a scale mounted at the bottom of each AMS. Body weights were averaged over a 24 h period and stored in T4C. All cows were fitted with a neck collar fixed with a mounted identification transponder that housed the cow's activity and rumination monitors (Qwes-HR; Lely, Maassluis, The Netherlands). Cow activity was measured by the accelerometer as the number of electronic impulses in 2 h intervals triggered by changes in acceleration due to head and neck movements. The rumination logger continuously recorded the minutes spent ruminating during a 24 h period in 2 h intervals as validated by Ambriz-Vilchis et al. (2015). Automatic readers were mounted in each AMS for data transfer from the accelerometer and logger at each milking. Transponders were replaced when their reliability fell below 85%.

Activity monitor data was used to determine d to first estrus. The first estrus after 20 DIM was defined as the day when estrus was detected by measurement of physical activity or visual observation by farm staff. An estrus was recorded if activity crossed the program's heat probability threshold with a concomitant decrease in rumination time and/or milk production. Along with AI service outcomes, days in milk at first service and DIM at conception were exported from T4C.

Health programs were managed by farm staff and they maintained health records throughout the study. Animals that were diagnosed and treated for clinical mastitis, displaced abomasum (DA), or culled during the first 100 DIM were removed from the data set. Diagnosis, treatment, and culling of cows was at the veterinarian and owner's discretion.

2.4. Statistical analysis

Statistical analyses of production data (except for milk fatty acids) were performed separately for pp and mp cows. In order to independently investigate effects of CLA supplementation and any potential post-supplementation carry over effects the statistical analysis was performed for two periods: the supplementation period (0 DIM to the end of supplementation) and post-supplementation period (the day after the end of supplementation to 100 DIM). Daily milk yield, milk composition, milking frequency, body weight, and rumination time were condensed to weekly means and analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with repeated measures and first-order autoregressive covariance structure. The model accounted for fixed effects of treatment, wk, and treatment × wk interaction and the random effect of cow(treatment). If the treatment effect or the treatment × wk interaction was significant, the SLICE option was used to compare treatment differences at individual wk. Calving month and AMS pen were included as categorical explanatory variables in all repeated measures models; if not significant, these variables were removed and the models rerun. Milk fatty acid profile and vitamins were analyzed by PROC MIXED using a model that accounted for fixed effect of treatment and the random effect of cow(treatment).

Table 4
Least squares means for lactation performance measures during the supplementation and post-supplementation period[a].

	Supplementation[b]					Post-supplementation[c]				
	Treatment			*P*-value		Treatment			*P*-value	
Variable	CTL	CLA-ME	SEM	Trt	Trt*time	CTL	CLA-ME	SEM	Trt	Trt*time
Multiparous, n	97	99				97	99			
Milk yield, kg/d	44.7	46.6	0.64	0.04	0.21	52.4	54.0	0.74	0.12	0.43
FCM[d], kg/d	47.1	47.9	0.72	0.43	0.61	51.6	52.6	0.75	0.36	0.94
Milk net energy[e], MJ/d	136.4	138.9	2.05	0.37	0.51	148.5	150.6	1.80	0.43	0.90
Milk fat										
Content, g/kg	39.3	37.9	0.46	0.03	0.67	34.2	33.6	0.40	0.30	0.37
Yield, kg/d	1.71	1.71	0.03	0.97	0.88	1.79	1.80	0.03	0.71	0.93
Milk protein										
Content, g/kg	33.5	33.1	0.27	0.32	0.36	30.5	29.8	0.22	0.02	0.79
Yield, kg/d	1.46	1.50	0.022	0.19	0.53	1.59	1.60	0.023	0.70	0.56
Milk lactose										
Content, g/kg	47.9	47.9	0.08	0.92	0.49	47.8	48.0	0.09	0.18	0.18
Yield, kg/d	2.15	2.24	0.069	0.04	0.10	2.51	2.59	0.077	0.08	0.41
Milkings/d	3.0	3.0	0.064	0.59	0.49	2.8	2.8	0.053	0.85	0.40
Primiparous, n	38	38				38	38			
Milk yield, kg/d	29.9	30.9	0.66	0.29	0.02	31.3	34.2	0.91	0.03	0.07
FCM, kg/d	30.0	30.1	0.64	0.90	0.39	31.2	33.0	0.83	0.14	0.51
Milk net energy, MJ/d	87.4	87.9	1.88	0.87	0.26	90.4	96.2	2.43	0.11	0.60
Milk fat										
Content, g/kg	36.2	34.7	0.63	0.09	0.70	35.2	33.0	0.63	0.02	0.23
Yield, kg/d	1.05	1.04	0.024	0.58	0.84	1.09	1.12	0.030	0.45	0.85
Milk protein										
Content, g/kg	32.9	32.4	0.37	0.29	0.78	31.3	31.0	0.33	0.43	0.40
Yield, kg/d	0.97	0.98	0.022	0.64	0.03	0.98	1.06	0.062	0.04	0.56
Milk lactose										
Content, g/kg	48.3	48.4	0.13	0.89	0.92	48.8	48.8	0.17	0.74	0.86
Yield, kg/d	1.44	1.49	0.073	0.30	0.02	1.53	1.67	0.099	0.03	0.03
Milkings/d	2.1	2.1	0.035	0.99	0.68	1.9	2.0	0.051	0.42	0.55

[a] Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME) or received no supplement (CTL).
[b] Data represent daily values condensed to weekly means collected during the supplementation period.
[c] Data represent daily values condensed to weekly means collected after the supplementation period through 100 DIM.
[d] Fat corrected milk, calculated based on NRC (2001): Milk fat yield (kg/d) × 16.216 + milk yield (kg/d) × 0.4324.
[e] Calculated based on NRC (2001): Milk yield (kg/d) × [0.0929 × milk fat content (g/100 g) + 0.0563 × milk protein content (g/100 g) + 0.0395 × milk lactose content (g/100 g)].

The interval from calving to first estrus, first service, and conception in the first 100 DIM were evaluated by PROC LIFETEST of SAS 9.4. Survival analysis was conducted to evaluate the effect of treatment on DIM at first estrus, first service, and conception. Right censoring occurred for animals that left the herd due to live culling or death, or did not conceive before 100 DIM. Kaplan Meier survival analysis curves were constructed to illustrate the rate at which animals were inseminated and conceived. The effect of treatment on hazard of first estrus, first service, and conception during the first 100 DIM was evaluated using PROC PHREG of SAS 9.4 by creating Cox proportional hazard models. The model included treatment and season as categorical explanatory variables. Season was not significant and it was therefore removed and the model rerun. Animals that were not eligible for breeding were removed from the reproduction data analysis.

Values reported are least square means and standard errors of the mean unless otherwise stated. The significance level for treatment effect was predefined at $P \leq 0.05$ and trends toward significance when $0.05 < P \leq 0.10$ for all analyses.

3. Results

A total of 78 animals were removed from the data set for treatment of DA, clinical mastitis, or removal from the herd per farm standard operating procedure. Performance data for the supplementation and post-supplementation period are presented in Table 4.

3.1. Multiparous animal production

During the 30 d supplementation period, CLA-ME cows produced 2 kg/d more milk ($P = 0.04$) compared to CTL. During that time milk fat content was decreased ($P = 0.03$) by 0.14 percentage units which represents a 3.6% decrease for animals

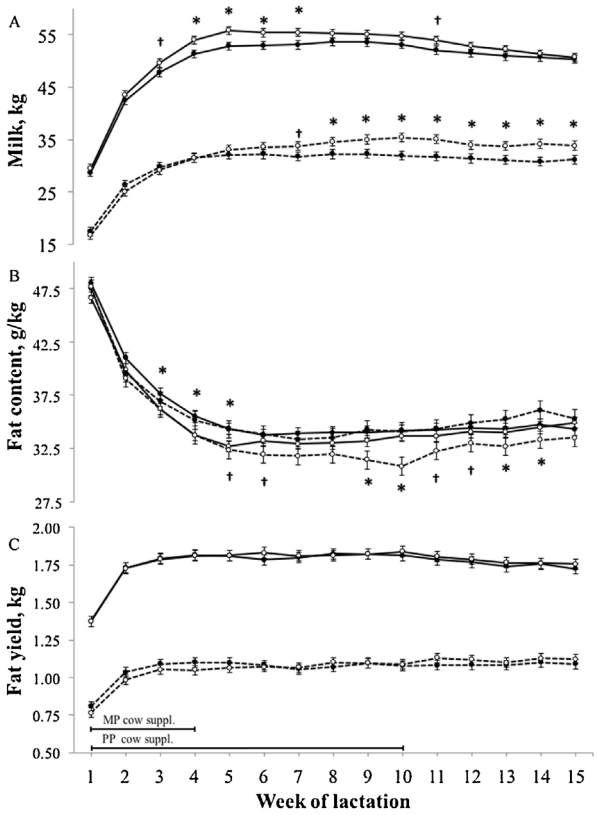

Fig. 1. Temporal changes in average daily milk production (A), milk fat content (B), and milk fat yield (C) by wk of lactation for multiparous (MP; solid; n = 198) and primiparous (PP; dashed n = 77) animals. Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME; open circles) or received no supplement (CTL; closed circles). Supplementation (suppl.) and post-supplementation (p-suppl.) periods were analyzed separately but are displayed together here. Main effect of treatment during suppl. for MP animals was $P = 0.04$ for panel A, $P = 0.03$ for panel B, and $P = 0.97$ for panel C. During p-suppl., main effect of treatment was $P = 0.12$ for panel A, $P = 0.30$ for panel B, and $P = 0.71$ for panel C for MP animals. For PP animals, main effect of treatment was $P = 0.29$ for panel A, $P = 0.09$ for panel B, and $P = 0.58$ during suppl. During p-suppl., main effect of treatment was $P = 0.03$ for panel A, $P = 0.02$ for panel B, and $P = 0.45$ for panel C in PP animals. Treatment × wk interactions for MP animals during suppl. and p-suppl. were $P > 0.15$. For PP animals treatment × wk interactions for panel A were $P = 0.02$ during suppl. and $P = 0.07$ during p.suppl., and $P > 0.15$ for panels B and C during suppl. and p-suppl. Symbols denote differences at individual wk based on SLICE effects as significance ($P \leq 0.05$; *) and trends toward significance ($0.05 < P \leq 0.10$; †). In panel B, symbols above the trend lines correspond to MP and below to PP animals.

supplemented with CLA; however, milk fat yield was unchanged ($P = 0.97$) compared to CTL. No difference was observed in daily milk protein content ($P = 0.32$) or yield ($P = 0.19$) during the supplementation period between CTL and CLA-ME. Daily milk lactose content was not changed ($P = 0.92$) by CLA supplementation, but daily lactose yield was increased ($P = 0.04$) and a treatment × wk interaction ($P = 0.10$) was detected. There was no difference in 3.5% FCM yield ($P = 0.43$) or milk net energy ($P = 0.37$) between CTL and CLA-ME during the supplementation period (Table 4).

During the post-supplementation period, daily milk yield, milk fat content, and milk fat yield did not differ between CTL and CLA-ME (Table 4). While daily milk protein content was decreased ($P = 0.02$) in CLA-ME, no difference ($P = 0.70$) was observed in milk protein yield compared to CTL during the 70 d post-supplementation period. Milk lactose content was not different ($P = 0.18$) in CLA-ME compared to CTL; however, lactose yield tended to increase ($P = 0.08$) with CLA supplementation. Yield of 3.5% FCM and milk net energy did not differ between CTL and CLA-ME during the post-supplementation period.

Temporal patterns for milk yield, milk fat content, and milk fat yield during the first 100 DIM are shown in Fig. 1. The yield of milk was similar for CTL and CLA-ME during the first 2 wk post-partum. Increased milk production was observed after wk 2 and persisted throughout the supplementation and into the post-supplementation period for animals supplemented with CLA. Treatment group differences in milk fat content appeared throughout the supplementation period for CLA-ME and persisted during the first wk of the post-supplementation period (Fig. 1).

3.2. Primiparous animal production

Daily milk yield was similar between CTL and CLA-ME during the 70 d supplementation period, but a treatment × wk interaction ($P = 0.02$) was detected. Animals supplemented with CLA tended to have decreased ($P = 0.09$) milk fat content, but

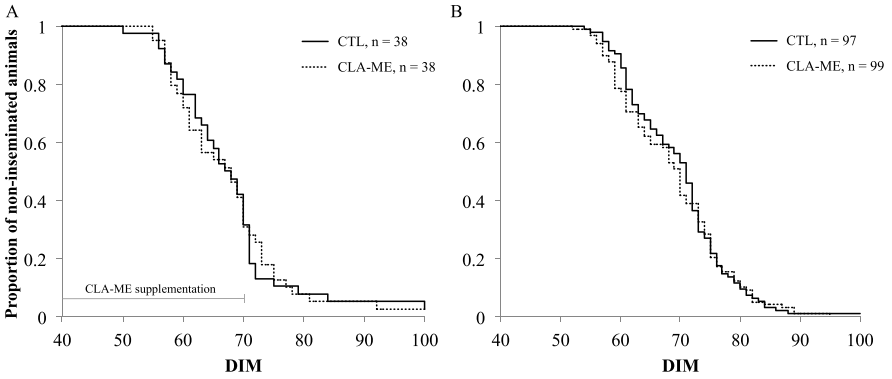

Fig. 2. Survival analysis curves illustrating the effect of treatment on interval from calving to first service during the first 100 DIM for primiparous (PP; A) and multiparous (MP; B) animals. Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME; open circles) or received no supplement (CTL; closed circles). Mean ± SE DIM at first service for PP animals were 67.0 ± 1.3 (P = 0.98) for CTL and 67.3 ± 1.5 for CLA-ME and for MP animals were 69.6 ± 0.8 (P = 0.82) for CTL and 68.9 ± 0.9 for CLA-ME. Median (95% CI) DIM at first service for PP animals were 67.5 (63–70) d for CTL and 68 (61–70) d for CLA-ME (P = 0.92), and for MP were 71 (67–72) d for CTL and 70 (65–71) d for CLA-ME (P = 0.44). Hazard ratios (95% CI) were 1.0 (0.6–1.6) for PP animals (P = 0.97) and 1.03 (0.8–1.4) for MP animals (P = 0.83).

no difference (P = 0.58) in daily milk fat yield was observed during that time. Content and yield of milk protein and lactose were similar between CTL and CLA-ME; however, yield of both components demonstrated a treatment-by-wk interaction (P < 0.05). In the supplementation period, 3.5% FCM yield and milk net energy were similar between CTL and CLA-ME (Table 4).

During the 30 d post-supplementation period, daily milk yield was increased (P = 0.03) by 3 kg/d in CLA-ME compared to CTL. In the same period, milk fat content was decreased (P = 0.02) by 0.22 percentage units, which represents a 6.25% decrease for CLA-ME; however, no change (P = 0.45) was detected in daily milk fat yield between treatment groups. While daily milk protein and lactose content were not altered during the post-supplementation period, yield of both components was increased (P < 0.05) in CLA-ME compared to CTL and a treatment × interaction was detected (P = 0.03) for lactose yield. Both 3.5% FCM yield and milk net energy were unchanged during the post-supplementation period.

Milk yield was similar between CTL and CLA-ME during the first 6 wk of the supplementation period (Fig. 1). Milk production was increased (P < 0.05) in CLA-ME compared to CTL from wk 8 through the end of the recorded post-supplementation period. Treatment group differences in milk fat content were not observed until later in the supplementation period and persisted throughout the recorded post-supplementation period (Fig. 1).

3.3. Vitamins and milk fatty acid composition

No difference was detected between treatment groups for milk concentrations of vitamin A (407.9 vs. 409.6 ± 30.6 ng/mL, CTL and CLA-ME respectively, P = 0.97) and vitamin E (0.257 vs. 0.298 ± 0.067 ug/mL, CTL and CLA-ME respectively, P = 0.66). Treatment effects on the reported proportions of individual milk fatty acids and fatty acid groups are shown in Table 5. The proportion of *trans*-10, *cis*-12 was increased (P = 0.03) in CLA-ME cows compared to CTL.

3.4. Body weight, rumination, and reproduction

A summary of average body weight and time spent ruminating on a daily basis for each treatment group during the supplementation and post-supplementation period is shown in Table 6. Body weight and daily rumination minutes did not differ between CTL and CLA-ME during either period for multiparous or primiparous animals (Table 6).

No effect of CLA supplementation was observed (P = 0.42) on the interval from calving to first estrus for multiparous (39.5 ± 1.2 vs. 40.3 ± 1.3 d, CTL and CLA-ME respectively) or primiparous (35.3 ± 1.3 vs. 36.5 ± 1.7 d, P = 0.49) animals, and median d to first estrus were not different between treatments for both multiparous (36; 95% CI = 35–40 vs. 37; 95% CI = 36–39 d, CTL and CLA-ME respectively, P = 0.78) and primiparous (36; 95% CI = 32–38 vs. 35; 95% CI = 32–36 d, P = 0.88) animals. The risk of first estrus was not affected by treatment for multiparous [hazard ratio (HR) = 0.89; 95% CI = 0.7–1.2], P = 0.44] and primiparous (HR = 0.90; 95% CI = 0.6–1.4, P = 0.67) animals.

Treatment did not affect the interval from calving to first service for multiparous (69.6 ± 0.8 vs. 68.9 ± 0.9 d, CTL and CLA-ME respectively, P = 0.82) and primiparous (67.0 ± 1.3 vs. 67.3 ± 1.5 d, P = 0.98) animals, nor the median d to first service for multiparous (71; 95% CI = 67–72 vs. 70; 95% CI = 65–71 d, CTL and CLA-ME respectively, P = 0.44) and primiparous (67.5; 95% CI = 63–70 vs. 68; 95% CI = 61–70 d, P = 0.92) animals. The rate of first service (Fig. 2) was not affected by treatment for multiparous (HR = 1.03; 95% CI = 0.8–1.4, P = 0.83) and primiparous (HR = 1.0; 95% CI = 0.6–1.6, P = 0.97) animals.

Supplementation with CLA did not affect calving to conception interval (85 ± 1.5 vs. 86 ± 1.5 d, CTL and CLA-ME respectively, P = 0.42), risk of conception during the first 100 DIM (HR = 0.86; 95% CI = 0.6–1.3, P = 0.43), and median DIM at

Table 5
Treatment effect on milk fatty acid composition.

	Treatment[a]			
Fatty acid, g/100 g total fatty acids	CTL	CLA-ME	SEM	*P*-value
4:0	4.96	4.97	0.18	0.95
6:0	2.12	2.17	0.17	0.82
8:0	1.03	1.05	0.12	0.86
10:0	2.10	2.19	0.29	0.82
10:1 *cis*-9	0.11	0.10	0.01	0.77
11:0	0.048	0.053	0.01	0.76
12:0	2.21	2.33	0.30	0.78
13:0	0.080	0.092	0.015	0.57
13:0 iso	0.011	0.014	0.003	0.36
13:0 *anteiso*	0.021	0.019	0.004	0.65
14:0	7.76	8.43	0.62	0.45
14:0 iso	0.063	0.065	0.007	0.86
14:1 *cis*-9	0.47	0.41	0.03	0.20
15:0	0.80	0.92	0.09	0.35
15:0 iso	0.13	0.15	0.01	0.29
15:0 *anteiso*	0.29	0.33	0.03	0.22
16:0	25.8	26.3	0.50	0.50
16:0 iso	0.24	0.23	0.02	0.88
16:1 *cis*-9	1.85	1.56	0.17	0.21
17:0	0.76	0.80	0.05	0.48
17:0 iso	0.31	0.34	0.02	0.25
17:0 *anteiso*	0.46	0.50	0.02	0.16
17:1 *cis*-9	0.38	0.36	0.03	0.56
18:0	13.4	14.0	0.48	0.36
18:1 *cis*-9	25.1	22.8	1.71	0.34
other 18:1	4.17	4.32	0.21	0.62
18:2 *cis*-9, *cis*-12	2.02	2.06	0.12	0.82
18:2 *cis*-9, *trans*-11	0.34	0.38	0.02	0.24
18:2 *trans*-10, *cis*-12	<0.001	0.017	0.005	0.03
18:3 *cis*-9, *cis*-12, *cis*-15	0.45	0.50	0.02	0.18
20:0	0.14	0.15	0.007	0.14
20:1 *cis*-11	0.058	0.055	0.003	0.44
20:2 *cis*-11, *cis*-14	0.09	0.08	0.016	0.44
20:3 *cis*-8, *cis*-11, *cis*-14	0.057	0.065	0.003	0.13
22:0	0.04	0.04	0.004	0.59
24:0	0.16	0.17	0.008	0.46
Other	2.01	2.03	0.07	0.88
MUFA	32.2	29.6	1.79	0.31
PUFA	2.96	3.09	0.13	0.46
SFA	62.9	65.3	1.82	0.35
Trans FA	3.19	3.44	0.23	0.45
Summation[b]				
de novo	20.7	21.7	1.57	0.68
C16	27.9	28.1	0.50	0.80
Preformed	53.5	52.6	1.68	0.68
Desaturation index				
14:1 *cis*-9/(14:0 + 14:1 *cis*-9)	0.061	0.048	0.007	0.18
16:1 *cis*-9/(16:0 + 16:1 *cis*-9)	0.067	0.056	0.006	0.19
18:1 *cis*-9/(18:0 + 18:1 *cis*-9)	0.65	0.62	0.017	0.21

[a] Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME) or received no supplement (CTL).
[b] Fatty acids are categorized according to origin: *de novo* represents sum of straight even-chain FA from C6 to C14, preformed represents sum of odd-chain FA and all FA with chain length of 18C or more including even chain isoand *anteiso* FA, C16 represents sum of 16C FA excluding 16:0 iso.

conception (91; 95% CI = 80–98 vs. 98; 95% CI = 86–100 d, $P = 0.51$) for multiparous animals. In primiparous animals, CLA supplementation did not affect the calving to conception interval (86.9 ± 2.7 vs 80.3 ± 2.5 d, CTL and CLA-ME respectively, $P = 0.15$) nor the risk of conception during the first 100 DIM (HR = 1.56; 95% CI = 0.9–2.9, $P = 0.15$). No difference was detected between median DIM at conception for primiparous animals, as median DIM at conception were 78 d (95% CI = 70–97 d) for CLA-ME and not reached for CTL because 50% of CTL animals did not become pregnant before 100 DIM. Supplementation with CLA did not affect conception rate at first service for multiparous (42 vs. 36%, CTL and CLA-ME respectively, $P = 0.36$) animals but tended to increase ($P = 0.10$) the first service conception rate in primiparous animals (35 vs 53%, CTL and CLA-ME respectively).

Table 6
Least squares means for body weight, and rumination minutes during the supplementation and post-supplementation period[a].

	Supplementation[b]					Post-supplementation[c]				
	Treatment			*P*-value		Treatment			*P*-value	
Variable	CTL	CLA-ME	SEM	Trt	Trt*time	CTL	CLA-ME	SEM	Trt	Trt*time
Multiparous										
Body weight, kg	693	695	6.6	0.82	0.015	692	695	6.3	0.77	0.80
Rumination, min	458	477	10.1	0.16	0.52	464	475	11.0	0.47	0.52
Primiparous										
Body weight, kg	556	561	8.8	0.68	0.86	586	589	8.6	0.75	0.99
Rumination, min	418	426	14.6	0.70	0.87	420	448	17.7	0.27	0.73

[a] Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME) or received no supplement (CTL).
[b] Data represent daily values condensed to weekly means collected during the supplementation period.
[c] Data represent daily values condensed to weekly means collected after the supplementation period through 100 DIM.

4. Discussion

The transition to lactation period poses a substantial metabolic and physiological challenge for dairy cows. Animals typically enter a negative energy balance (NEB) as energy intake is insufficient to meet maintenance and milk synthesis requirements (Drackley, 1999). Mitigating this NEB requires a shift in nutrient partitioning that necessitates coordination of metabolism to ensure an adequate supply of nutrients to meet energy requirements and any maladaptation increases the risk for metabolic disorders. Improved energy balance could allow the cow to adapt to lactation without compromising health, reproduction, or milk production (Griinari and Bauman, 2003).

Milk fat is the most energetically expensive component of milk, requiring half of the total energy required for milk synthesis and up to 35% of a dairy cow's net energy intake (Bauman and Currie, 1980). Reducing milk fat synthesis can reduce the energy required for milk production (NRC, 2001). *trans*-10, *cis*-12 CLA, a documented milk fat depressing fatty acid (Bauman and Griinari, 2003), offers a unique nutritional opportunity to modulate the energy metabolized for milk fat synthesis. By limiting uptake of preformed FA and decreasing *de novo* FA synthesis in mammary tissue (Hussein et al., 2013), supplementing CLA could spare energy for increased milk production, growth, or maintenance. Some studies have reported no improvement in energy status during CLA supplementation, with spared energy being partitioned to increase milk volume (Bernal-Santos et al., 2003; Hutchinson et al., 2012), whereas others indicated decreases in milk energy output and therefore improved calculated energy balance (Odens et al., 2007; Hutchinson et al., 2011). Cows experiencing CLA-induced milk fat depression during early lactation had reduced milk fat content by 10–26% and reduced fat yield by 7.5–23% (Bernal-Santos et al., 2003 Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; Moallem et al., 2010; Hutchinson et al., 2011, 2012; Schlegel et al., 2012). When cows were supplemented for longer than 60 d of lactation and milk fat content was decreased by less than 16%, milk yield was increased (Bernal-Santos et al., 2003; Moallem et al., 2010; Hutchinson et al., 2012; Schlegel et al., 2012).

Based on these previously reported patterns of decreased milk fat and increased milk volume, we hypothesized that CLA supplementation would alter energy metabolism to increase milk production during the transition to lactation period. Cows were supplemented with CLA pre-calving to account for the major metabolic changes during transition and to immediately induce milk fat depression after calving. Postpartum supplementation was designed to supplement through the transition period and to the time of predicted peak in milk yield which was 30 DIM for multiparous cows and 70 DIM for primiparous cows. Data was recorded until 100 DIM to determine the possible carry over effects of long-term CLA supplementation. Cows were supplemented with 100 g of the CLA methyl ester product because a similar dose of the *trans*-10, *cis*-12 CLA isomer was previously shown to sustainably induce milk fat depression, but not sacrifice milk yield (Moore et al., 2004). Examining the proportion of FA in milk confirmed that the CLA supplement supplied the isomers post-ruminally for uptake by the mammary gland. At 7 ± 1 DIM the proportion of *trans*-10, *cis*-12 was increased in the milk FA of CLA-ME cows (Table 5).

It is generally accepted that *trans*-10, *cis*-12 CLA induces milk fat depression by coordinately down-regulating the expression of key enzymes involved in mammary gland lipogenesis, lipid desaturation, and uptake of preformed FA (Bauman et al., 2011; Hussein et al., 2013). The proportion of *trans*-10, *cis*-12 CLA was greater in cows supplemented with CLA-ME in this study. Only a numerical increase in *cis*-9, *trans*-11 CLA was observed in CLA-ME cows; however, this could be due to coinciding increases in supply of supplemental *cis*-9, *trans*-11 CLA and decreased desaturation rate of *trans*-11 18:1. The purpose of milk sample analysis within this study was to confirm CLA isomers were being absorbed by the animal and analyses may have been inadequately powered to detect differences in specific fatty acids or fatty acid groups. Although, no difference was detected in the summation of FA considered to have *de novo* or preformed origin, the desaturation index for three major saturated lipids (14:0, 16:0, and 18:0), and the proportion of total monounsaturated FA, were all numerically decreased in cows supplemented CLA. Of the preformed FA, the proportion of both 20:0 and 20:3 *n*-6 were numerically increased in CLA-ME cows. Others have observed significant increases in the proportion of 20:0 with CLA supplementation (Kay et al., 2006; Hutchinson et al., 2012; Dallaire et al., 2014). Only a numerical increase in the proportion of 20:3 *cis*-8, *cis*-11, *cis*-14

was observed here; however, others have reported a decrease in the proportion of this FA with abomasal infusion of CLA (Dallaire et al., 2014). This could also be attributed to the marginal decrease in milk fat content experienced by the cows supplemented in this study. A similar trial to the one described here detected a CLA-induced decrease in the proportion of *de novo* and preformed FA, but did so in cows experiencing reduced milk fat content by 17% (Hutchinson et al., 2012). Nonetheless, the observed changes in milk FA of CLA-ME cows suggests the CLA isomers were acting upon the mammary gland to alter the lipid profile.

In the current work, multiparous animals supplemented with CLA had increased milk yield during the supplementation period and numerically produced more milk during the post-supplementation period. For primiparous animals, milk yield was not increased until the post-supplementation period, but a treatment × time interaction was observed during the supplementation period (Table 4) and SLICE effects determined the increase in milk yield began in wk 8 of lactation (Fig. 1). Milk fat content was not reduced until wk 3 of lactation for CLA-ME multiparous animals and wk 9 for primiparous animals (Fig. 1). Previous studies have also observed a delay between the start of CLA supplementation and a detectable reduction in milk fat content (Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007). It has been suggested this may be due to insensitivity or unresponsiveness of cows during early lactation because key enzymes and biochemical pathways are altered to support the onset of lactation (Castañeda-Gutiérrez et al., 2005). Milk fat content was maximally reduced in wk 5 and returned to contents similar to CTL cows by wk 6 of lactation for multiparous animals, only 2 wk after supplementation stopped, but increased milk yield continued until wk 7. Depressed milk fat content was delayed in primiparous CLA-ME as well and did not occur until wk 9, near the end of the supplementation period, and carried into the post-supplementation period (Fig. 1). Previous trials have also observed sustained reductions in milk fat content for variable lengths of time after CLA supplementation ceased (Castañeda-Gutiérrez et al., 2005; Hutchinson et al., 2012). Supplementing cows with 5 or 18 g/d of *trans*-10, *cis*-12 CLA until wk 9 of lactation reduced milk fat content until wk 16 (Hutchinson et al., 2012) or wk 11 (Castañeda-Gutiérrez et al., 2005), respectively. The prolonged decrease in milk fat content could reflect lasting effects of altered energy metabolism, incorporation and later release of *trans*-10, *cis*-12 CLA from adipose tissue stores, or altered gene expression of lipogenic enzymes in the mammary gland.

As milk yield increased and milk lactose content remained constant, milk lactose yield was increased (Table 4). This is to be expected, as lactose is the major osmoregulator of water uptake by the mammary gland and therefore controls milk fluid volume (Linzell, 1972). Increased lactose yield has been similarly observed in a previous study examining CLA supplementation during early lactation (Hutchinson et al., 2012). Similarly, milk protein content was decreased in multiparous CLA-ME during the post-supplementation period, but yield remained unchanged because milk yield increases compensated for the depleted content. Conversely, the increased milk yield during the post-supplementation period and unaffected protein content collectively increased daily protein yield for primiparous CLA-ME. The treatment × time interaction for milk protein content during the supplementation period suggests that CLA supplementation was altering protein yield in primiparous animals during that period as well. Others have also observed an increase in milk protein yield during CLA supplementation and for a residual period after the end of supplementation (Medeiros et al., 2010).

The increased milk yield was adequate enough to offset the depressing effects of *trans*-10, *cis*-12 CLA on milk fat content such that no difference was detected in daily milk fat yield between the CTL and CLA-ME groups for either primiparous or multiparous animals. The increase in milk yield compared to CTL suggests that in every kg of milk secreted energy could be repartitioned so that more kg of milk, at a reduced milk fat content, could be produced. This translated into the same daily milk fat yield between treatment and controls. Lipogenic enzymes use acetate and butyrate as well as energy and reducing equivalents to synthesize FA *de novo* in the mammary gland (Bauman and Davis, 1974). When milk fat synthesis is down regulated by CLA supplementation, energy and substrates are spared from metabolism and the major carbon source saved is acetate (Urrutia et al., 2015). When cows were infused with a calculated equivalent of acetate estimated from glucose spared during milk fat depression, milk and fat yields were increased, indicating that acetate supply affects milk production and fat synthesis (Urrutia et al., 2015). If acetate supply was increased by CLA supplementation enough to spare glucose, lactose synthesis from the glucose supply could have increased fluid milk volume. A previous experiment demonstrated that CLA supplementation decreased endogenous glucose production but increased lactose output and plasma glucose concentration compared to controls, by a mechanism attributed to diminished glucose catabolism for milk fat synthesis (Hötger et al., 2013). Although the current work was not designed to determine the mechanism of CLA's action, the nutrient shifts described above may have supported an increase in milk volume while maintaining milk fat yield.

Despite the increase in milk and lactose yield, no differences were observed in daily milk energy output with CLA supplementation for multiparous cows. In primiparous animals, the combined increase in milk, lactose, and protein yield numerically increased milk energy output in primiparous CLA-ME during the post-supplementation period. Treatment with CLA has either not altered (Bernal-Santos et al., 2003; Medeiros et al., 2010; von Soosten et al., 2011; Hutchinson et al., 2012) or reduced milk energy output in previous reports (Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; Hutchinson et al., 2011). If no difference in energy intake was observed, the numerically greater energy output could imply an increase in efficiency, unfortunately the study design did not allow for measuring intake and subsequent efficiency, but this should be considered in future studies.

Energy repartitioned from milk fat synthesis in each kg of milk could also be used for milk components, growth, or maintenance rather than milk volume. Even though body condition score (BCS) was not measured, body weights taken multiple times per day may account for rumen fill and offer a more accurate tool to access growth or changes in condition. No difference was observed in body weight between CTL and CLA-ME animals in either period suggesting that the treatment groups

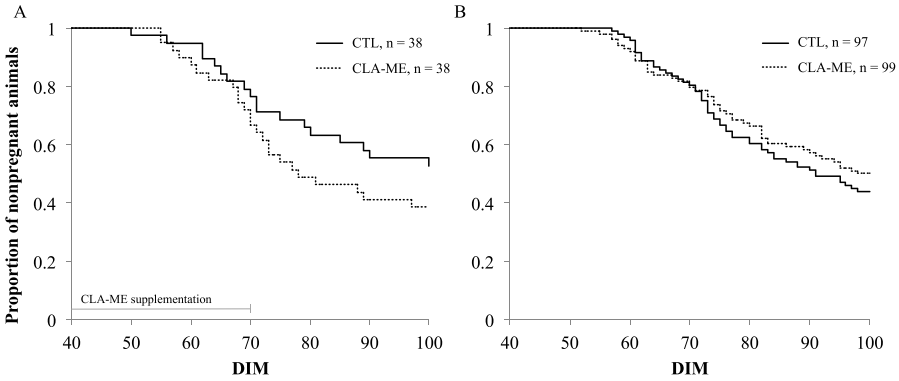

Fig. 3. Survival analysis curve illustrating the effect of treatment on time to pregnancy during the first 100 DIM for primiparous (PP; A) and multiparous (MP; B) animals. Animals were supplemented with 100 g/d of a lipid encapsulated CLA methyl ester product (Lutrell® Pure) mixed 50:50 with soybean meal from −21 pre-calving through 30 DIM for multiparous cows and 70 DIM for primiparous cow (CLA-ME; open circles) or received no supplement (CTL; closed circles). Mean ± SE calving to conception intervals for PP animals were 86.9 ± 2.7 ($P = 0.15$) for CTL and 80.3 ± 2.5 for CLA-ME. For MP animals, mean ± SE calving to conception intervals were 85.0 ± 1.5 for CTL and 86.0 ± 1.5 for CLA-ME ($P = 0.42$). Median (95% CI) calving to conception interval for PP animals was 78 (70–97) d for CLA-ME and not reached for CTL. For MP animals, median (95% CI) calving to conception intervals were 91 (80–98) d for CTL and 98 (86–100) d for CLA-ME ($P = 0.51$). Hazard ratios (95% CI) were 1.56 (0.9–2.9) for PP animals ($P = 0.15$) and 0.86 (0.6–1.3) for MP animals ($P = 0.43$).

lost and gained condition at similar rates. Other trials that measured body weight and BCS during CLA supplementation have reported either no difference in condition and growth (Castañeda-Gutiérrez et al., 2005; Moallem et al., 2010), or gains in body weight and condition of CLA supplemented cows (Bernal-Santos et al., 2003; Hutchinson et al., 2012). Still, others have reported reduced body weight and BCS loss during the early fresh period in CLA supplemented cows (Odens et al., 2007; Hutchinson et al., 2011).

It is not possible to determine calculated energy balance because DMI was not measured here. The energy repartitioned from milk fat synthesis to increase yield of milk and other components in primiparous CLA-ME may have been enough to stimulate an increase in energy intake and or tissue mobilization to account for the numerically increased milk energy output. The added fat, protein, and energy supplied by the 100 g of SBM and CLA supplement would not have been enough to account for this additional milk energy output alone. Previous studies have reported variable effects of CLA supplementation on DMI including decreased (Moallem et al., 2010; von Soosten et al., 2011), unchanged (Bernal-Santos et al., 2003; Moore et al., 2004; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; von Soosten et al., 2011), or increased intake (Shingfield et al., 2004). A *meta*-analysis of voluntary intake during CLA infusion revealed a 1.5 kg/d decrease in DMI. Spared energy from milk fat synthesis during CLA supplementation may have improved energy balance and the reduced energy requirement reduced DMI in that study. Short-term abomasal infusion of CLA decreased milk fat content and yield by 34% and 38% respectively and decreased lipogenic gene expression in mammary tissue, but increased expression of lipogenic enzymes and lipid synthesis regulators in adipose tissue (Harvatine et al., 2009). This suggests that adipose tissue was in a net lipogenic state and lipid storage increased during CLA-induced milk fat depression, possibly indicating improved energy balance. This may be an indirect effect of sparing energy at the level of the mammary gland, but it remains to be determined if the effect is independent of energy balance, especially during long-term CLA supplementation that induces marginal milk fat depression.

As control animals were not feed an isoenergetic source of rumen-inert fat, it is possible that the added energy provided by the CLA supplement may have contributed to increased milk yields during the supplementation period. However, the estimated 5 MJ/d of supplemental energy provided by the CLA and SBM would have contributed less than 3% additional diet energy which is much lower than the variation of energy intake observed in previous studies that supplemented CLA and isoenergetic control diets and measured DMI (Moore et al., 2004; Odens et al., 2007; Harvatine et al., 2009). If the treatment effects observed here were only attributed to the supplemental energy, it is not likely that milk composition would have been altered during the post-supplementation period as observed in both primiparous and multiparous CLA-ME animals, especially without a change in milk energy output (Fig. 1 and Table 4). Additionally, sustained alteration in milk composition after CLA supplementation supports the hypothesis that the mechanism of CLA action is through altering regulation of milk synthesis (Bauman et al., 2011). The effects of CLA supplementation in the current experiment are similar to what has been observed previously in studies that fed CLA and compared to an isoenergetic control (Bernal-Santos et al., 2003; Moore et al., 2004; Castañeda-Gutiérrez et al., 2005; Kay et al., 2006; Odens et al., 2007; Medeiros et al., 2010; Moallem et al., 2010; Hutchinson et al., 2011), or fed CLA and compared effects to a non-supplemented control group (Hutchinson et al., 2012).

Improved calculated energy balance during the transition period is associated with improved reproductive efficiency (Beam and Butler, 1999; Webb et al., 1999; Butler, 2001). Multiparous CLA-ME animals did not differ from CTL in DIM at, or the risk of first estrus, first service, and conception (Figs. 2 and 3), nor did conception rate at first service differ between multiparous treatment groups. However, primiparous animals supplemented with CLA had a tendency for increased conception rate at first service compared to CTL. This most likely contributed to the numerically shorter interval from calving to conception and numerically increased risk for conception in the first 100 DIM in primiparous CLA-ME animals. Improved reproductive efficiency with CLA supplementation has been previously reported (Bernal-Santos et al., 2003; Castañeda-

Gutiérrez et al., 2005). Both of these trials reported numerically improved calculated energy balance. Despite only observing 17 multiparous animals, a trial reported a trend for reduced interval to first ovulation and a numerical increase in conception rate of CLA supplemented cows (Bernal-Santos et al., 2003). In a larger study (n = 48) a higher proportion of cows supplemented with CLA became pregnant before 185 DIM (Castañeda-Gutiérrez et al., 2005). A *meta*-analysis of the dietary effects of fat on fertility of dairy cattle reported a consistent numerical decrease in calving-to-conception interval with CLA supplementation (Rodney et al., 2015). A similar study to the one described here supplemented over 200 cows for the first 60 DIM but did not observe any effect of CLA on interval to first ovulation, first service, or conception rate (Hutchinson et al., 2012). It should be considered that that trial did not separate animals by parity and only supplemented 5 g of the *trans*-10, *cis*-12 CLA compared to the 10 g supplemented in the current work. Additionally, treatment and control cows observed in Hutchinson et al. (2012) were managed in a pasture-based system and may have deposited more CLA in milk as has been observed in pasture vs. TMR-feeding systems (Kelly et al., 1998; O'Callaghan et al., 2016). The limited number of adequately powered studies makes it difficult to definitively conclude a positive impact of CLA supplementation on reproductive efficiency and necessitates more research. Conception may have been improved in multiparous CLA-ME animals if supplementation had lasted through the breeding period; however, the lack of difference in measures of reproductive efficiency between multiparous treatment groups suggests that milk yield was increased without adversely affecting reproductive efficiency.

The feeding and management system used in this study enabled accurate and reliable automatic supplementation of CLA to individual animals and demonstrated that CLA supplementation during the transition period temporarily reduced milk fat content in lactating animals on a commercial dairy. Milk fat is economically important for producers, especially in markets where premiums are paid for milk components. For this reason, milk fat depression is often undesirable. This study demonstrated that a nominal reduction in milk fat content can be associated with an increase in milk volume and maintain milk fat production. Duration of CLA supplementation may dictate the combined effect on milk and fat yield.

5. Conclusions

Feeding rumen-protected CLA mixtures may have beneficial applications, including as a strategy to increase milk and protein yield in order to maintain or improve performance during the transition to lactation period. This study demonstrated that supplementing mixed isomers of CLA at a rate of 20 g/d effectively reduced milk fat content but concurrently increased milk yield and maintained milk fat yield in multiparous and primiparous animals in a commercial setting. Despite increased milk yield, reproductive efficiency was maintained in multiparous animals and first-service conception rate tended to be improved in primiparous animals.

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