RUNNING HEAD: LE-CLA AND ENERGY STATUS

# Effects of Lipid Encapsulated-Conjugated Linoleic Acid supplementation on milk production, bioenergetic status and indicators of reproductive performance in lactating dairy cows

**Ian Hutchinson,*# Michael J de Veth,†[2] Catherine Stanton,‡ Richard J Dewhurst,§ Pat Lonergan,# Alex C O Evans,# and Stephen T Butler*[1]**

* Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

† BASF SE, 67056 Ludwigshafen, Germany

‡ Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland

§ Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland

# School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

[2] Present address: Department of Animal Science, The University of Tennessee, Knoxville, TN.

[1]Corresponding author: stephen.butler@teagasc.ie

## ABSTRACT

Conjugated linoleic acid (CLA) reduces mammary milk fat synthesis in a dose dependant manner. Our objective was to determine the effects of lipid encapsulated

CLA (LE-CLA) supplementation on milk production, reproductive performance and metabolic responses in lactating dairy cows fed a grass-silage based diet. Seventy-two Holstein-Friesian cows (32 primiparous and 40 multiparous) were used in a completely randomized block design. Cows received either 60 g per day of LE-CLA or 60 g per day of calcium salts of palm fatty acids (CSFA; control) from parturition until 60 days in milk. The LE-CLA contained a 50:50 mix of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, resulting in a daily intake of 6 g per day of each isomer. Milk production and dry matter intake (DMI) were recorded daily, and blood samples were collected 3 times per week. Blood samples were analysed for circulating concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), insulin and insulin-like growth factor-I (IGF-I). Progesterone was measured in blood samples collected after the first postpartum insemination. Ovarian ultrasound examinations commenced at 8-10 days postpartum and were carried out 3 times a week until first ovulation. The LE-CLA treatment resulted in decreased milk fat concentration, with consequent improvements in energy balance and body condition score (BCS). The peak concentration of NEFA in blood was reduced by LE-CLA, but circulating concentrations of insulin, glucose, IGF-I, BHBA and progesterone were not affected. There was no effect of LE-CLA supplementation on the postpartum interval to first ovulation. Services per conception tended to be reduced. The reduction in milk energy output and improvement in energy status and BCS in LE-CLA supplemented cows provides a strong rationale for further studies with greater cow numbers to test effects on reproductive performance.

Key words: conjugated linoleic acid, reproduction, milk fat, energy balance.

During early lactation in dairy cattle, the energy required for maintenance and milk energy output exceed dietary energy intake (Bell, 1995; Grummer, 1995) resulting in negative energy balance (NEB) and consequent body reserve mobilisation. Numerous reports have indicated that the duration and extent of NEB delay the onset of cyclicity and reduce the likelihood of conception (Beam & Butler, 1999; Diskin *et al.*, 2003) and also predispose the cow to health problems such as fatty liver and ketosis (Drackley, 1999). Efforts to overcome early postpartum NEB via dietary means have logically attempted to increase the energy density of the diet being fed. However, this approach has been largely unsuccessful. For instance, addition of fat to the diet increases the energy density of the diet, but is often associated with a modest reduction in DMI such that total energy intake remains unaffected (Staples *et al.*, 1998). Alternatively, where additional energy is ingested, this may be partitioned to increased milk production such that net energy balance is not improved (Santos *et al.*, 2008).

Conjugated linoleic acids (CLA) are geometric and positional isomers of linoleic acid, and are normally produced in the rumen as intermediates in the biohydrogenation of linoleic acid to stearic acid (Bauman & Griinari, 2000). *Trans*-10, *cis*-12 CLA is an isomer of CLA that inhibits milk fat synthesis (Baumgard *et al.*, 2000), and has been demonstrated to reduce mammary milk fat synthesis in a dose-dependent manner (de Veth *et al.*, 2004). This phenomenon has been observed in cows consuming both pasture (Kay *et al.*, 2006; Mackle *et al.*, 2003) and TMR diets (Bernal-Santos *et al.*, 2003; Odens *et al.*, 2007) and at varying stages of lactation. However, the dose of *trans*-10, *cis*-12 CLA necessary to evoke milk fat depression (MFD) immediately postpartum (36.9 g/d; Moore *et al.*, 2004) was found to be greater than the dose necessary in established lactation (8.8 g/d; Perfield et al., 2002).

Fat is energetically the most expensive component of milk; daily milk fat secretion in early lactation cows represents up to 35% of net energy intake (Bauman & Currie, 1980). The milk fat depressing effects of *trans*-10, *cis*-12 CLA could therefore be used as a management tool to temporarily reduce milk energy output. Importantly, milk volume and milk protein concentration are not decreased by CLA supplementation, and MFD caused by *trans*-10, *cis*-12 CLA is reversible, with milk fat content returning to similar levels as control groups at the termination of supplementation (Castaneda-Gutierrez *et al.*, 2005).

Beneficial effects of CLA supplementation on fertility indices have been reported, including trends towards decreased interval to first ovulation, elevated plasma progesterone during the early luteal phase, increased plasma IGF-I and increased pregnancy rate (Bernal-Santos *et al.*, 2003; Castaneda-Gutierrez *et al.*, 2007; Castaneda-Gutierrez *et al.*, 2005); this was recently confirmed in a meta-analysis of 5 published studies (de Veth *et al.*, 2009). The objective of the present study was to determine the effects of feeding a concentrate pellet supplement containing lipid encapsulated CLA (LE-CLA) on early lactation milk production, bioenergetic and metabolic status, and reproductive indices in dairy cows consuming a grass-silage based diet.

## MATERIALS AND METHODS

### *Animals and treatments*

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland, in accordance with the Cruelty to Animals Act (Ireland 1897) and the European Community Directive 86/609/EC. Forty multiparous Holstein-Friesian cows were blocked on the basis of expected calving

date, previous lactation milk yield, and body condition score (BCS), and 32 primiparous Holstein-Friesian cows were blocked on the basis of expected calving date, BCS and bodyweight. Treatments were initiated during a 4-month period. Cows were then randomly assigned to receive 80 g per day of LE-CLA (Lutrell Pure; BASF SE, Ludwigshafen, Germany) or 60 g per day of calcium salts of palm fatty acids (CTL; Megalac; Volac Ltd., Hertfordshire, UK) from parturition until 60 days in milk. To facilitate administration of the treatments, the fatty acid supplements were incorporated into concentrate pellets such that 2 kg of concentrate contained 60 g of LE-CLA or CSFA supplements, and concentrate pellets were fed using automatic feeders. A preliminary investigation indicated that the pelleting process resulted in a 13.4% loss of the *trans*-10, *cis*-12 CLA isomer recovered in the concentrate. To maintain the minimum targeted dose of 6 g per day of *trans*-10, *cis*-12 CLA, the incorporation of LE-CLA was increased to 80 g/day. The LE-CLA contained a 50:50 mix of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, resulting in a daily intake of 6.9 g per day of each isomer after adjusting for losses incurred during pelleting. The protection technology for LE-CLA has been previously described (Perfield *et al.*, 2004). The cows were housed in a free stall cubicle shed and managed as a single group of animals throughout the study period. Three cows were removed from the study due to illnesses unrelated to the experimental treatments.

Individual DMI was measured daily from 3 weeks prior to parturition to 13 weeks postpartum using the Griffith-Elder Mealmaster feeding system (Griffith Elder & Co Ltd, Suffolk, UK). Cows were housed on a straw bed for a variable period of 1 to 4 days around the time of parturition. Weekly samples of the feeds offered were dried and ground, and composited on a monthly basis for nutrient analysis. The dry matter, crude protein, NDF, ash and oil content of the feed samples were analysed as

described by McNamara *et al.* (2003). The ingredient composition of the prepartum and postpartum basal diets and the concentrate supplements are outlined in Table 1.

Cows were fed daily at 0900 h, and were allowed access to their fat supplements from 0900 to 1700. Cows consumed their allocated fat supplements in a single meal, and the allotted time allowed ample access time for all cows. Following parturition, cows were milked twice daily at 0700 and 1600 h. Milk yield was recorded daily at morning and evening milkings using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat, protein and lactose) was determined on a weekly basis from successive morning and evening milk samples by automated infra-red absorption analysis using a Milkoscan 605 (Foss Electric, Hillerod, Denmark). Cow body weight (kg) and BCS (Lowman *et al.*, 1976) were recorded once per week from parturition until week 13 of lactation.

Energy balance was estimated as the difference between energy intake and the sum of energy requirements for maintenance and milk production, using the French net energy system (Jarrige, 1989). This system uses unité fourragère lait (UFL) as the unit of net energy, which is equivalent to 1 kg of standard air-dried barley. The following equations were used to determine the energy required for maintenance and output in milk (O' Mara, 1997);

energy required for maintenance (UFL/d) = 1.4 + 0.6 BW/100;

energy requirement for milk (UFL/kg of milk) = 0.0054 FC + 0.0031 PC + LC -0.015;

where FC = fat concentration (%), PC = protein concentration (%), and LC = lactose concentration (%).

During the period from day 21 before calving to day 28 postpartum, blood samples were collected on three days per week (Monday, Wednesday and Friday). Thereafter, blood samples were collected every 2 weeks until 10 weeks postpartum. In

addition, blood samples were collected from each cow 3 times per week for 21 days after the first postpartum insemination for progesterone analysis. All blood samples were collected from the coccygeal vessels into vacutainers containing lithium heparin (Becton Dickinson, Plymouth, United Kingdom) between 0700 and 0800 h after the morning milking but before feeding. The samples were immediately centrifuged at 2000 × *g* for 15 min. The plasma was decanted into 1.5 mL tubes, sealed with an airtight cap, and stored at -20 °C until analysis.

***Hormone and metabolite analysis***

Blood samples collected that were closest in proximity to days -14, -7, 0, 7, 14, 21, 28, 42, 56 and 70 relative to parturition were analysed for circulating concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), insulin, and insulin-like growth factor-I (IGF-I). For example, mean actual sampling day for 'Day 7' samples was day 7, with a range from day 5 to day 9. For 'Day 14' samples, mean actual sampling day was day 14, with a range from day 12 to 16. All other sample days followed a similar pattern. Plasma glucose, NEFA and BHBA concentrations were determined by enzymatic colorimetry using an ABX Pentra autoanalyzer (ABX Mira, Montpellier, France) and the appropriate enzymatic kits (glucose kits supplied by ABX Mira, Montpellier, France; NEFA kits supplied by Wako Chemicals GmbH, Neuss, Germany; BHBA kits supplied by Randox Laboratories Ltd., Crumlin, Co. Antrim, Northern Ireland). Plasma progesterone and insulin concentrations were measured using time-resolved fluoroimmunoassays (AutoDELFIA, PerkinElmer Life and Analytical Science, Turku, Finland) with the appropriate kits (Unitech BD Ltd., Dublin, Ireland). Circulating concentrations of IGF-I were determined by radioimmunoassay following ethanol:acetone:acetic acid

extraction as previously described (Butler et al., 2004). The inter- and intra-assay coefficients of variation for progesterone, insulin and IGF-I were 5.0% and 4.6%, 4.4% and 5.0%, and 10.9% and 10.9%, respectively. The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was used to assess insulin sensitivity in lactating dairy cows. The index values were calculated using the formula RQUICKI = 1/[log(glucose) + log(insulin) + log(NEFA)] as described by Holtenius & Holtenius (2007).

***Reproductive measurements and breeding management***

Ovarian structures were examined by linear array ultrasonography using a 7.5-MHz transrectal transducer (Aloka SSD-900; Aloka Ltd., Tokyo, Japan). Ultrasound examinations commenced on day 8 to 10 postpartum, and were carried out on 3 days per week (Monday, Wednesday and Friday) until first ovulation. Follicles were considered to be dominant when a diameter of >10 mm was reached in the absence of other large, growing follicles (Savio et al., 1990). Cysts were defined as anovulatory follicles >25 mm in diameter that persisted for at least 10 d in the absence of a corpus luteum (Garverick, 1997). Initiation of breeding commenced on a calendar mating start date, and continued for 15 weeks. Tail paint was used as a heat detection aid, and all cows were inseminated using AI following observation of standing estrus, or removal of tail paint, or both. Pregnancy diagnoses were carried out at 30 to 36 and 60 to 66 d post-insemination. Visualization of a fluid-filled horn and a viable embryo were used for positive identification of pregnancy.

***Milk fatty acid analysis***

Milk samples were collected at 28 DIM (S.D. ± 2.2 days) and analysed for FA composition using GLC, as described by Mohammed *et al.* (2009). Briefly, milk lipids were extracted using a chloroform/methanol/water mixture and methylated using $NaOCH_3$/methanol. The Fatty Acid Methyl Esters (FAME) were analyzed using a Hewlett Packard Model 5890 Series II GLC, and identified by comparison with a GLC reference standard (#463) spiked with a mixture of 4 positional conjugated linoleic acid (CLA) isomers (#UC-59M), 21:0, 23:0, and 26:0 obtained from Nu-Chek Prep Inc, Elysian, Minnesota, USA. Identification of 16:1, 18:1, and 20:1 isomers was based on available isomers in the GLC reference standard (#463), comparison with published reports, and based on principles of silver-ion separation. Individual FAME were reported as a percentage of total FAME.

***Statistical analysis***

All statistical analyses were carried out using SAS (SAS System Inc., Cary, NC). Daily measurements of milk yield, dry matter intake, and energy balance were collapsed into weekly means. A test for normality was performed on all the blood analyte data and R-QUICKI values. Each of the variables had a non-normal distribution, and were log-transformed prior to analysis to generate a normal distribution. Milk production, milk composition, dry matter intake, cow body weight, body condition score, energy balance and blood analyte data were analysed using mixed models with repeated measures, using the satterthwaite adjustment to calculate denominator degrees of freedom. The appropriate covariance structure for each repeated measures analysis was identified based on Akaike's Information Criterion (AIC) model fit statistic. Measurements made during the final 3 weeks prepartum were included as covariates in the models for glucose, NEFA, BHBA, IGF-I, insulin,

dry matter intake, cow body weight, body condition score and calculated energy balance. Parity and calving day of year were included as adjustment variables in all repeated measures models; if non-significant, these variables were removed and the models were re-run. Peak NEFA concentration and energy balance nadir after day 1 of lactation were identified for all cows. Milk fatty acid composition, energy balance nadir, timing of energy balance nadir and peak NEFA concentrations were analysed using mixed models procedures with treatment as a fixed effect, block as a random effect, and calving day of year included as an adjustment variable. All values reported are least squares means and SEM. Conception rate data were analysed using the Chi-square test, and values reported are treatment means. All other reproductive performance data were analysed using mixed models procedures. Data were considered significant when $P < 0.05$, and a trend declared when $P < 0.1$.

## RESULTS

### *Milk production, energy balance and BCS*

The milk production results are summarized in Table 2 and Figure 1. Milk fat concentration was reduced by LE-CLA (treatment effect: $P < 0.001$), but there was no treatment by time interaction. The greatest reduction in milk fat concentration occurred at week 8 postpartum (15.7%) at the end of the supplementation period. Milk fat yield was also reduced by up to 15.2% during this period ($P = 0.008$). Supplementation with LE-CLA ceased at 60 DIM, and thereafter milk fat concentration in LE-CLA cows began to return toward concentrations similar to the control group after week 10 postpartum. Supplementation with LE-CLA had no effect on milk yield, the concentration or yield of milk protein, or the concentration or yield

of milk lactose. Mean daily milk energy output tended (P = 0.06) to be lower for cows on the LE-CLA treatment.

Dry matter intake was not affected by LE-CLA supplementation (17.2 kg/d vs. 17.3 kg/d CTL and LE-CLA respectively; Figure 2). Mean energy balance was greater for the cows on the LE-CLA treatment (P < 0.001; Figure 2). Energy balance nadir tended to be less severe in LE-CLA cows (-4.29 UFL/d vs. -3.18 UFL/d CTL and LE-CLA respectively, P = 0.09), but the timing of the NEB nadir did not differ (2.2 weeks after parturition vs. 1.9 weeks after parturition CTL and LE-CLA respectively). Consequently mean postpartum BCS also tended to be greater for LE-CLA cows (P = 0.09; Figure 2). Body condition score declined in both treatments for the first 3 weeks of lactation (BCS change week 1 to week 3 CTL: 3.20 to 3.01, P = 0.01; LE-CLA: 3.18 to 3.00, P = 0.03). Thereafter LE-CLA cows did not lose any further BCS whereas control cows continued to mobilise body reserves until week 5 (BCS change week 3 to week 5 CTL: 3.01 to 2.83, P = 0.02; LE-CLA: 3.00 to 3.00, P = 0.99). This resulted in a lower mean nadir BCS in CTL cows during weeks 5 to 8 (week 5 to 8 BCS: 2.96 vs. 2.83; treatment by time, P < 0.05). Cow body weight (BW) was not affected by treatment, and there was no treatment by time interaction. Mean BW during the treatment period were 555.8 kg and 551.51 kg (CTL and LE-CLA, respectively).

***Milk fatty acid analysis***

LE-CLA supplementation reduced the proportion of most short and medium chain fatty acids in milk fat compared to the CTL animals (Table 4). The proportion of C16:0 in milk fat was also decreased by LE-CLA supplementation (P = 0.01). The proportion of *trans*-10, *cis*-12 CLA in milk fat was increased by LE-CLA

supplementation (P = 0.01), as were the proportions of other long chain fatty acids including C18:0, *cis*-9 18:1, C18:3 n-3 and C20:0 (all P < 0.03). The proportion of *cis*-9, *trans*-11 CLA tended to be increased (P = 0.06) by LE-CLA supplementation.

***Metabolites and metabolic hormones***

Plasma metabolite data are illustrated in Figure 3. Plasma glucose concentrations were not affected by LE-CLA. No differences in mean circulating NEFA were detected using repeated measures analysis. Peak circulating concentrations of NEFA during the postpartum period were greater in CTL cows compared to LE-CLA cows (0.69 ± 0.06 vs. 0.49 ± 0.06 mmol/L, P = 0.004), but the mean day when peak circulating NEFA was observed did not differ between treatments (6.8 ± 1.1 vs. 5.8 ± 1.1 DIM). Plasma BHBA was not affected by treatment. Treatment by time interactions were not observed for any of the blood metabolites. Mean circulating concentrations of insulin and the calculated R-QUICKI index values were not different between treatments (Figure 4). There were no effects of treatment or treatment by time interaction on circulating IGF-I concentrations. However, a significant (P < 0.05) effect of parity was detected (128.6, 120.2 and 108.3 ng/ml for parity 1, 2 and ≥3 respectively). A treatment by parity interaction (P < 0.05) was observed, whereby LE-CLA supplementation appeared to increase circulating IGF-I in parity 1 cows (135.1 vs. 122.1 ng/ml) and parity ≥3 cows (117.2 vs. 99.4 ng/ml), but resulted in reduced circulating IGF-I in parity 2 cows (111.6 vs. 128.8 ng/ml).

***Reproductive performance***

Reproductive performance data are summarized in Table 3. There was no difference between treatments in the postpartum interval to onset of cyclicity (21.0 ± 2.5 vs. 23.3 ± 2.6 days postpartum, CTL and LE-CLA, respectively). Conception rate to first or second service were not affected by LE-CLA supplementation, but the number of services per conception tended to be reduced (P = 0.07). Calving to service interval and calving to conception interval did not differ between the treatments (Table 3).

Circulating concentrations of progesterone during the first 21 days after insemination were not affected by LE-CLA supplementation, regardless of whether or not a pregnancy was successfully established.

## DISCUSSION

In the present study, LE-CLA supplementation during the first 60 days postpartum reduced milk fat concentration, with maximal MFD (15.7%) observed at 8 weeks after parturition. The energy spared by reducing milk fat synthesis resulted in improved calculated energy balance status and BCS. The current study did not have had enough animals to allow a robust statistical appraisal of effects of LE-CLA on reproductive performance (a Power calculation based on the conception rates to first service observed in the current study with $\alpha = 0.05$ and $1-\beta = 0.9$ indicated ~323 animals per treatment would be required). However the improvements in energy status and BCS provide a potential mechanism to improve fertility; this needs to be tested in a commercial trial where larger numbers of cows can be enrolled in a study.

In previous studies that commenced supplementation with CLA before or immediately after parturition, MFD did not occur immediately postpartum. Bernal-Santos *et al.* (2003) fed 0 or 8.8 g/d *trans*-10, *cis*-12 CLA and Castaneda-Gutierrez *et*

*al.* (2005) fed 0, 8.8 or 18.3 g/d *trans*-10, *cis*-12 CLA; they did not observe CLA-induced MFD until approximately the third week of lactation. In the current study, we observed a significant treatment effect on milk fat concentration, but there was no treatment by time interaction, which would suggest that LE-CLA reduced milk fat concentration at all timepoints. Moore *et al.* (2004) suggested that the mammary gland is less sensitive to LE-CLA-induced MFD during very early lactation. This was also observed by Castaneda-Gutierrez *et al.* (2005) and they speculated that mammary responsiveness and/or sensitivity to *trans*-10, *cis*-12 CLA during the early postpartum period is altered to support lactation. In the current study, the supplement provided an intake of 6.9 g/day of *trans*-10, *cis*-12 CLA. This dose is similar to the study of Castaneda-Gutierrez *et al.* (2007), but considerably less than Kay *et al.* (2006) and Odens *et al.* (2007) who used doses of 20.9 g/day and 29.3 g/day, respectively. It is important to note that milk fat is economically important to dairy farmers. Extreme MFD may be undesirable, especially in regions where milk is primarily used for manufacturing purposes. The amount of LE-CLA supplement fed in the present study was chosen to induce sufficient MFD to potentially improve energy status, while not markedly decreasing income from milk.

Supplementation with LE-CLA improved calculated energy balance, and this was reflected in improved BCS in the LE-CLA supplemented cows compared to CTL animals. To our knowledge, improved BCS in early lactation dairy cows in response to CLA supplementation has not previously been reported. The improvement in energy balance can be directly attributed to MFD, as DMI and milk yield did not differ between CTL and LE-CLA treatment groups. This is consistent with previous research that has identified beneficial effects of feeding LE-CLA on energy balance indices (Odens *et al.*, 2007; Kay *et al.*, 2007). A recent report on the effects of *trans*-

10, *cis*-12 LE-CLA on adipose tissue supports the effect of LE-CLA on BCS observed in the current study. Harvatine *et al.* (2009) investigated the expression of lipid-related genes in adipose tissue during *trans*-10, *cis*-12 CLA induced MFD in mid-lactation dairy cows (i.e., positive energy balance). Those authors identified a net energy excess caused by a decrease in milk fat synthesis, which was accompanied by increased expression of genes involved in the uptake, synthesis, desaturation and transport of FA in adipose tissue, consistent with enhanced energy partitioning to body fat stores during CLA supplementation (Harvatine *et al.*, 2009). In the current study, the cows were in early lactation NEB, but it is plausible that energy spared through the effects of LE-CLA on milk energy output could affect metabolic pathways in adipose tissue to reduce lipid mobilisation in the same manner observed by Harvatine *et al*. (2009).

Reduced severity of NEB should reduce the demand to mobilise adipose tissue reserves. We did not detect an effect of CLA on mean circulating NEFA concentrations during the supplementation period, but peak circulating NEFA concentrations were lower in the CLA supplemented cows compared to the CTL cows. The literature is inconsistent with regard to the effect of LE-CLA on circulating NEFA concentrations, with some reports identifying a reduction in NEFA in LE-CLA supplemented cows (Odens *et al.*, 2007) while others reported no effect (Kay *et al.*, 2007; Castaneda-Gutierrez *et al.*, 2005). Collectively, the results of the present and earlier studies indicate that (i) CLA acts to lower NEFA only if supplementation is initiated at or before parturition; (ii) reductions in NEFA are not detectable if circulating NEFA is already low at the start of the study (mid- to late-lactation cows in positive energy balance); and (iii) ability to detect an effect of CLA treatment on NEFA with a small number of cows per treatment is hindered by the high variability

382 that is typically observed in circulating NEFA concentrations during the transition
383 period. Lower circulating NEFA during the transition period may result in reduced
384 liver uptake of NEFA and subsequent esterification to triglycerides, resulting in a
385 beneficial effect on liver health status (Drackley, 1999).

386 We observed no differences in plasma glucose concentrations for LE-CLA
387 supplemented cows compared with CTL cows. The literature is inconsistent on the
388 effect of CLA on plasma glucose in early lactation dairy cows. Odens *et al.* (2007)
389 observed greater plasma glucose in cows supplemented with a high dose of CLA, but
390 other studies have failed to detect a similar effect (Bernal-Santos *et al.*, 2003; Kay *et*
391 *al.*, 2006; Castaneda-Gutierrez *et al.*, 2005). Odens *et al.* (2007) attributed the greater
392 circulating glucose concentrations to a possible decrease in peripheral tissue insulin
393 sensitivity. However, studies where an insulin tolerance test was used to measure
394 insulin sensitivity in cows either abomasally infused with CLA (Baumgard *et al.*,
395 2002a) or fed LE-CLA (de Veth *et al.*, 2006) reported no difference in the fractional
396 rate of change in circulating glucose following insulin administration. Similarly, LE-
397 CLA supplementation did not affect the R-QUICKI index values in the present study,
398 again suggesting insulin sensitivity was not affected. It is possible that the effects of
399 CLA on peripheral tissue insulin responsiveness are subtle, and cannot be detected
400 using techniques such as the insulin tolerance test or the R-QUICKI index in lactating
401 dairy cows.

402 The current study was not adequately powered to detect effects on
403 reproduction related variables. Accordingly, we did not observe any effect on the
404 majority of the fertility measurements, with the exception of a trend for a reduced
405 number of services per conception (for all cows, pregnant and non-pregnant) in LE-
406 CLA supplemented cows. A limited number of published reports, all with low

numbers of cows per treatment, have indicated statistical trends for beneficial effects of CLA on fertility indices (Castaneda-Gutierrez *et al.,* 2005, n = 18/treatment; Bernal-Santos *et al.,* 2003, n = 15/treatment; Castaneda-Gutierrez *et al.,* 2007, n = 15/treatment). Beneficial effects included trends for reduced postpartum interval to first ovulation, numerical improvements in conception rate, and reduced number of services per conception. The data from these three studies were combined with data from Mann *et al.* (2007) and de Veth *et al.* (2005) in a recent meta-analysis (de Veth *et al.,* 2009) which indicated that the optimal dose for advancing the postpartum onset of cyclicity was 8 g/d *trans*-10, *cis*-12 CLA; interval to first postpartum ovulation was reduced by 8 days in cows fed this dose (de Veth *et al.*, 2009). The optimal dose for improving conception was 10 g/d *trans*-10, *cis*-12 CLA; this dose increased the probability of cows becoming pregnant (72% vs. 91%) and reduced the time to conception (143 DIM vs. 105 DIM) compared to cows receiving no supplement (de Veth *et al.*, 2009). In the current study, we did not observe an effect of LE-CLA feeding on postpartum onset of cyclicity; resumption of luteal activity occurred early postpartum in both the LE-CLA and CTL treatment groups. The effects of LE-CLA on conception related variables in the present study are generally in agreement with previous reports (Bernal-Santos *et al.*, 2003; Castaneda-Gutierrez *et al.*, 2007; Castaneda-Gutierrez *et al.*, 2005) and the recent meta-analysis (de Veth *et al.*, 2009).

Insulin-like Growth Factor-I is a polypeptide hormone, the concentration of which in circulation is correlated with nutritional status (McGuire *et al.*, 1995) and reproductive performance in dairy cattle (Taylor *et al.*, 2004). Castaneda-Gutierrez *et al.* (2007) previously reported greater circulating IGF-I in LE-CLA supplemented cows compared to control cows, despite not detecting an effect on energy balance. In the current study, the effect of LE-CLA on circulating IGF-I concentrations appeared

to differ by parity, but similar treatment by parity interactions were not observed in the analysis of energy balance and BCS. Further work is necessary to determine if the effect of CLA on circulating IGF-I truly differs by parity.

Progesterone is a steroid hormone that is critical for successful establishment of pregnancy. In a synchronized cycle, Castaneda-Gutierrez *et al.* (2007) observed greater progesterone concentrations during the luteal phase in LE-CLA supplemented cows. In the current study, progesterone was measured for 21 days after insemination, but LE-CLA did not affect concentrations in cows that were subsequently diagnosed either pregnant or non-pregnant. The differences between the results of the current study and those of Castaneda-Gutierrez *et al.* (2007) in IGF-I and progesterone hormone profiles warrant further study.

The milk fatty acid results indicated that approximately 0.18 g of *trans*-10, *cis*-12 LE-CLA escaped biohydrogenation and was available for intestinal absorption, representing a transfer efficiency of 2.7%. Three other studies have fed LE-CLA supplements, reporting transfer efficiencies of 2.6 % (de Veth *et al.*, 2006), 4.8 % (Moallem *et al.*, 2010) and 7.9 % (Perfield *et al.*, 2004). The variation in transfer efficiencies between studies may partly reflect differences in the technology used to protect CLA in the different studies. The transfer efficiency in the present study was achieved despite pelleting the LE-CLA supplement, which could potentially lead to degradation of the lipid encapsulation protection due to heating during the pelleting process. This may enhance the potential for practical application of the LE-CLA supplement used in the current study. Though it is likely that the pelleting process had some detrimental effect on rumen protection, under the conditions that LE-CLA was pelleted in this study, the supplement remained efficacious at reducing milk fat content.

The changes in milk fat composition in response to LE-CLA supplementation in the present study – i.e., decreasing proportions of short and medium chain fatty acids and increasing proportions of long chain fatty acids – are consistent with other reports (Mackle *et al.*, 2003; Perfield *et al.*, 2002; Castaneda-Gutierrez *et al.*, 2005). Collectively, these studies indicate that the reduction in milk fat synthesis during CLA supplementation is due to reductions in both the uptake of preformed fatty acids and, to a greater extent, de novo mammary synthesis of short chain fatty acids. Baumgard *et al.* (2002b) demonstrated that *trans*-10, *cis*-12 CLA decreases milk fat synthesis through a reduction in mRNA expression for key enzymes involved in fat synthesis in the mammary gland, as well as enzymes involved in the uptake and transport of circulating fatty acids.

## CONCLUSIONS

Supplementation with LE-CLA induced MFD in early lactation dairy cows, with no effect on milk yield, or the yield of milk protein or lactose. The reduction in milk fat output resulted in greater net energy balance in LE-CLA supplemented cows, and this was reflected in improved BCS. The number of services per conception tended to be lower in LE-CLA supplemented cows, although no other reproduction variables were changed. The current study goes beyond previous work by demonstrating effects on energy balance and BCS by feeding a small amount of supplemental LE-CLA in pelleted form. Because of the well established relationship between energy balance and reproduction in lactating dairy cows, and a recent meta-analysis indicating CLA can benefit reproduction, further work with a larger number of animals is necessary to fully determine the effects of LE-CLA on cow fertility.

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## REFERENCES


Bauman, D.E. & Currie, W.B. (1980) Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science 63(9), 1514-1529.

Bauman, D.E. & Griinari, J.M. (2000) Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science 70(1-2), 15-29.

Baumgard, L.H., Corl, B.A., Dwyer, D.A., Saebo, A. & Bauman, D.E. (2000) Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. American Journal of Physiology Regulatory Integrative and Comparative Physiology 278(1), R179-184.

Baumgard, L.H., Corl, B.A., Dwyer, D.A. & Bauman, D.E. (2002a) Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. Journal of Animal Science 80(5), 1285-1293.

Baumgard, L.H., Matitashvili, E., Corl, B.A., Dwyer, D.A. & Bauman, D.E. (2002b) Trans-10, cis-12 Conjugated Linoleic Acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. Journal of Dairy Science 85(9), 2155-2163.

Beam, S.W. & Butler, W.R. (1999) Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. Journal of reproduction and fertility. Supplement 54, 411.

Bell, A.W. (1995) Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73(9), 2804-2819.

Bernal-Santos, G., Perfield, J.W., II, Barbano, D.M., Bauman, D.E. & Overton, T.R. (2003) Production responses of dairy cows to dietary supplementation with Conjugated Linoleic Acid (CLA) during the transition period and early lactation. Journal of Dairy Science 86(10), 3218-3228.

Butler, S.T., Pelton, S.H. & Butler, W.R. (2004) Insulin increases 17β-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction 127(5), 537-545.

Castaneda-Gutierrez, E., Overton, T.R., Butler, W.R. & Bauman, D.E. (2005) Dietary supplements of two doses of calcium salts of Conjugated Linoleic Acid during the transition period and early lactation. Journal of Dairy Science 88(3), 1078-1089.

Castaneda-Gutierrez, E., Benefield, B.C., de Veth, M.J., Santos, N.R., Gilbert, R.O., Butler, W.R. & Bauman, D.E. (2007) Evaluation of the mechanism of action of Conjugated Linoleic Acid isomers on reproduction in dairy cows. Journal of Dairy Science 90(9), 4253-4264.

de Veth, M.J., Griinari, J.M., Pfeiffer, A.M. & Bauman, D.E. (2004) Effect of CLA on milk fat synthesis in dairy cows: Comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39(4), 365-372.

de Veth, M.J., van Straalen, W.M., Koch, W., Keller, T., Hayler, R. & Pfeiffer, A.M. (2005) Effect of CLA dose on milk production in early lactation cows. Journal of Dairy Science 88, 220-220.

de Veth, M.J., Castaneda-Gutierrez, E., Dwyer, D.A., Pfeiffer, A.M., Putnam, D.E. & Bauman, D.E. (2006) Response to Conjugated Linoleic Acid in dairy cows differing in energy and protein status. Journal of Dairy Science 89(12), 4620-4631.

de Veth, M.J., Bauman, D.E., Koch, W., Mann, G.E., Pfeiffer, A.M. & Butler, W.R. (2009) Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows. Journal of Dairy Science 92(6), 2662-2669.

Diskin, M.G., Mackey, D.R., Roche, J.F. & Sreenan, J.M. (2003) Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Animal Reproduction Science 78(3-4), 345-370.

Drackley, J.K. (1999) Biology of dairy cows during the transition period: The final frontier? Journal of Dairy Science 82(11), 2259-2273.

Garverick, H.A. (1997) Ovarian follicular cysts in dairy cows. Journal of Dairy Science 80(5), 995-1004.

Grummer, R.R. (1995) Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science 73(9), 2820-2833.

Harvatine, K.J., Perfield, J.W., II & Bauman, D.E. (2009) Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. Journal of Nutrition 139(5), 849-854.

Holtenius, P. & Holtenius, K. (2007) A model to estimate insulin sensitivity in dairy cows. Acta Veterinaria. Scandinavica. 49(1), 29.

Jarrige, J. (1989) In INRAtion v. 2.7: Microsoft computer program of ration formulation for ruminant livestockEdited by J. Agabriel, P. Champciaux & C. Espinasse. CNERTA, Dijon, France.

Kay, J.K., Roche, J.R., Moore, C.E. & Baumgard, L.H. (2006) Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture. Journal of Dairy Research 73(3), 367-377.

Kay, J.K., Mackle, T.R., Bauman, D.E., Thomson, N.A. & Baumgard, L.H. (2007) Effects of a supplement containing trans-10, cis-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. Journal of Dairy Science 90(2), 721-730.

Lowman, B.G., Scott, N. & Somerville, S.H. (1976) Condition scoring of cattle. East of Scotland College of Agriculture. Bulletin 6, 31.

Mackle, T.R., Kay, J.K., Auldist, M.J., McGibbon, A.K.H., Philpott, B.A., Baumgard, L.H. & Bauman, D.E. (2003) Effects of abomasal infusion of Conjugated Linoleic Acid on milk fat concentration and yield from pasture-fed dairy cows. Journal of Dairy Science 86(2), 644-652.

Mann, G.E., Lock, A.L., Bauman, D.E. & Kendall, N.R. (2007) Reproductive function in dairy cows fed a lipid encapsulated conjugated linoleic acid supplement. Journal of Dairy Science 90, 401-401.

McGuire, M.A., Bauman, D.E., Dwyer, D.A. & Cohick, W.S. (1995) Nutritional modulation of the Somatotropin/Insulin-Like Growth Factor system: Response to feed deprivation in lactating cows. Journal of Nutrition 125(3), 493-502.

McNamara, S., Butler, T., Ryan, D.P., Mee, J.F., Dillon, P., O'Mara, F.P., Butler, S.T., Anglesey, D., Rath, M. & Murphy, J.J. (2003) Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein-Friesian cows. Animal Reproduction Science 79(1-2), 45-56.

Moallem, U., Lehrer, H., Zachut, M., Livshitz, L. & Yacoby, S. (2010) Production performance and pattern of milk fat depression of high-yielding dairy cows supplemented with encapsulated conjugated linoleic acid. Animal 4(4), 641-652.

Mohammed, R., Stanton, C.S., Kennelly, J.J., Kramer, J.K.G., Mee, J.F., Glimm, D.R., O'Donovan, M. & Murphy, J.J. (2009) Grazing cows are more efficient

than zero-grazed and grass silage-fed cows in milk rumenic acid production. Journal of Dairy Science 92(8), 3874-3893.
Moore, C.E., Hafliger, H.C., Mendivil, O.B., Sanders, S.R., Bauman, D.E. & Baumgard, L.H. (2004) Increasing amounts of conjugated linoleic acid (CLA) progressively reduces milk fat synthesis immediately postpartum. Journal of Dairy Science 87(6), 1886-1895.
O' Mara, F. (1997) A Net Energy System for Cattle and Sheep. Belfield, Dublin 4, Ireland Department of Animal Science and Production, Faculty of Agriculture, University College Dublin.
Odens, L.J., Burgos, R., Innocenti, M., VanBaale, M.J. & Baumgard, L.H. (2007) Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. Journal of Dairy Science 90(1), 293-305.
Perfield, J.W., II, Bernal-Santos, G., Overton, T.R. & Bauman, D.E. (2002) Effects of dietary supplementation of rumen-protected Conjugated Linoleic Acid in dairy cows during established lactation. Journal of Dairy Science 85(10), 2609-2617.
Perfield, J.W., II, Lock, A.L., Pfeiffer, A.M. & Bauman, D.E. (2004) Effects of amide-protected and lipid-encapsulated Conjugated Linoleic Acid (CLA) supplements on milk fat synthesis. Journal of Dairy Science 87(9), 3010-3016.
Santos, J.E.P., Bilby, T.R., Thatcher, W.W., Staples, C.R. & Silvestre, F.T. (2008) Long chain fatty acids of diet as factors influencing reproduction in cattle. Reproduction in Domestic Animals 43, 23-30.
Savio, J.D., Boland, M.P., Hynes, N. & Roche, J.F. (1990) Resumption of follicular activity in the early post-partum period of dairy cows. Journal of Reproduction and Fertility 88(2), 569-579.
Staples, C.R., Burke, J.M. & Thatcher, W.W. (1998) Influence of supplemental fats on reproductive tissues and performance of lactating cows. Journal of Dairy Science 81(3), 856-871.
Taylor, V.J., Cheng, Z., Pushpakumara, P.G.A., Beever, D.E. & Wathes, D.C. (2004) Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Veterinary Record 155(19), 583-588.

620 Table 1. Ingredient and nutrient composition of the feeds offered

| **TMR ingredients (% of DM)** | **Prepartum** | **Postpartum** |
|---|---|---|
| Grass silage | 100 | 50 |
| Soya hulls | | 25 |
| TMR premix[1] | | 25 |
| **Nutrient composition of TMR** | | |
| DM (g/kg) | 234 | 364 |
| Net energy (UFL/kg DM)[2] | 0.75 | 0.91 |
| Ash (g/kg DM) | 84 | 74 |
| Crude protein (g/kg DM) | 157 | 175 |
| NDF (g/kg DM) | 536 | 462 |

| **Concentrate supplement (% as fed)** | **CTL** | **LE-CLA** |
|---|---|---|
| Barley | 20.0 | 20.0 |
| Citrus pulp | 26.1 | 26.1 |
| Maize gluten feed | 26.5 | 26.5 |
| Soyabean meal | 19.0 | 19.0 |
| CSFA | 4.4 | - |
| LE-CLA | - | 4.4 |
| Vitamins and minerals[3] | 4.0 | 4.0 |
| **Nutrient composition of concentrate** | | |
| DM (g/kg) | 879 | 879 |
| Net energy (UFL/kg DM) | 1.15 | 1.15 |
| Ash (g/kg DM) | 109 | 97 |
| Crude protein (g/kg DM) | 184 | 196 |
| NDF (g/kg DM) | 205 | 218 |
| Oil (acid hydrolysis) % | 35 | 54 |

621 [1]Ingredient composition: 60% rolled barley, 36% soyabean meal, 4% vitamins and minerals[3]

622 [2]UFL = unité fourragère lait; unit of net energy, equivalent to 1 kg of standard air-dried barley

623 [3]Vitamin and mineral mix: 15 g/kg dicalcium phosphate, 8 g/kg limestone flour, 5 g/kg salt, 2.5 g/kg
624 calcined magnesite, 80 mg manganous oxide, 200 mg copper sulphate, 125 mg zinc oxide, 18 mg
625 potassium iodate, 20 mg sodium selenite (4.6%), 10 mg cobalt sulphate, 8 MIU/t vitamin A, 2 MIU/t
626 vitamin D3, 15,000 IU/t vitamin E.

627

Table 2. Least squares means for milk yield and milk composition during the treatment period

| | Treatment[1] | | | P-value[4] | |
|---|---|---|---|---|---|
| | CTL | LE-CLA | S.E.M | Trt | Trt × Time |
| Milk yield (kg/day) | 26.5 | 26.5 | 0.9 | 0.9 | 0.8 |
| Milk fat | | | | | |
| % | 4.26 | 3.78 | 0.11 | <0.001 | 0.4 |
| kg/day | 1.08 | 0.98 | 0.04 | 0.008 | 0.5 |
| Milk protein | | | | | |
| % | 3.12 | 3.07 | 0.05 | 0.4 | 0.14 |
| kg/day | 0.81 | 0.78 | 0.03 | 0.15 | 0.7 |
| Milk lactose | | | | | |
| % | 4.63 | 4.62 | 0.03 | 0.7 | 0.6 |
| kg/day | 1.23 | 1.22 | 0.04 | 0.8 | 0.8 |
| Milk energy (UFL/day)[2] | 11.4 | 10.7 | 0.4 | 0.06 | 0.7 |
| Milk energy (Mcal/day)[3] | 19.4 | 18.3 | 0.7 | 0.06 | 0.7 |

[1] CTL = Control; LE-CLA = Lipid Encapsulated Conjugated Linoleic Acid

[2] UFL = unité fourragère lait; unit of net energy, equivalent to 1 kg of standard air-dried barley

[3] Milk energy (Mcal/day) = ((0.0929*Fat %) + (0.0563*Protein %) + (0.0395*Lactose %)) * Milk yield

[4] The effect of time was significant (P<0.05) for all variables

Table 3. Reproductive performance of cows on the CTL and LE-CLA supplements

| | Treatment[1] | | |
|---|---|---|---|
| | CTL | LE-CLA | P-value |
| Interval to 1st ovulation (days) | 21.0 (± 1.6) | 23.3 (± 1.7) | 0.6 |
| Dom. foll. diameter at 1st ovulation[2] (mm) | 15.4 (± 0.8) | 14.8 (± 0.8) | 0.5 |
| Wave number at 1st ovulation[3] | 1.48 | 1.52 | 0.9 |
| Conception rate to first service (%) | 38.9 (14/36) | 51.5 (17/33) | 0.3 |
| Conception rate to second service (%) | 66.7 (14/21) | 76.9 (10/13) | 0.7 |
| Services/conception for pregnant cows (n) | 1.63 (± 0.15) | 1.48 (± 0.14) | 0.3 |
| Services/conception for all cows (n) | 2.00 (± 0.15) | 1.72 (± 0.16) | 0.07 |
| Calving to service interval (days) | 81.1 (± 6.1) | 83.2 (± 6.5) | 0.7 |
| Calving to conception interval (days) | 111.0 (± 8.8) | 109.1 (± 8.6) | 0.8 |

[1] CTL = Control; LE-CLA = Lipid Encapsulated Conjugated Linoleic Acid

[2]The maximum diameter of the first postpartum ovulatory dominant follicle.

[3]The follicle wave when the first postpartum ovulation occurred.

638 Table 4. Milk fatty acid composition (g/100g total fatty acids) of cows on the CTL
639 and LE-CLA supplements at week 4 of lactation

| | Treatment[1] | | | |
|---|---|---|---|---|
| | CTL | LE-CLA | S.E.M | P-value |
| 4:0 | 3.36 | 3.58 | 0.067 | 0.03 |
| 6:0 | 1.93 | 1.80 | 0.035 | 0.03 |
| 8:0 | 1.11 | 0.98 | 0.029 | <0.01 |
| 10:0 | 2.40 | 2.04 | 0.087 | 0.01 |
| 10:1 | 0.28 | 0.22 | 0.013 | <0.01 |
| 11:0 | 0.06 | 0.04 | 0.005 | 0.02 |
| 12:0 | 2.75 | 2.28 | 0.103 | <0.01 |
| 12:1 | 0.07 | 0.06 | 0.008 | 0.69 |
| 13:0 | 0.10 | 0.09 | 0.005 | 0.23 |
| 14:0 | 9.95 | 8.98 | 0.235 | 0.01 |
| 14:1 | 1.09 | 0.90 | 0.037 | <0.01 |
| 15:0 | 1.15 | 1.08 | 0.027 | 0.07 |
| 16:0 | 30.90 | 28.62 | 0.650 | 0.01 |
| *cis*-9 16:1 | 1.42 | 1.43 | 0.062 | 0.98 |
| 17:0 | 0.71 | 0.77 | 0.015 | 0.02 |
| 17:1 | 0.35 | 0.39 | 0.012 | 0.01 |
| 18:0 | 8.44 | 9.68 | 0.352 | 0.02 |
| *trans*-11 18:1 | 1.40 | 1.49 | 0.072 | 0.37 |
| *cis*-9 18:1 | 17.59 | 20.22 | 0.697 | 0.01 |
| *cis*-11 18:1 | 0.43 | 0.47 | 0.030 | 0.44 |
| 18:2n-6 | 1.64 | 1.69 | 0.051 | 0.50 |
| 18:3n-3 | 0.51 | 0.56 | 0.017 | 0.02 |
| 20:0 | 0.12 | 0.15 | 0.006 | <0.01 |
| *cis*-9, *trans*-11 CLA | 0.51 | 0.58 | 0.026 | 0.06 |
| *trans*-10, *cis*-12 CLA | 0.01 | 0.02 | 0.003 | 0.01 |
| 20:1 | 0.09 | 0.10 | 0.005 | 0.25 |
| 20:4n-6 | 0.10 | 0.11 | 0.004 | 0.25 |
| All <C:16 | 23.09 | 20.94 | 0.475 | <0.01 |
| All C:16 | 32.08 | 29.55 | 0.652 | <0.01 |
| All >C16 | 33.37 | 37.67 | 1.040 | <0.01 |

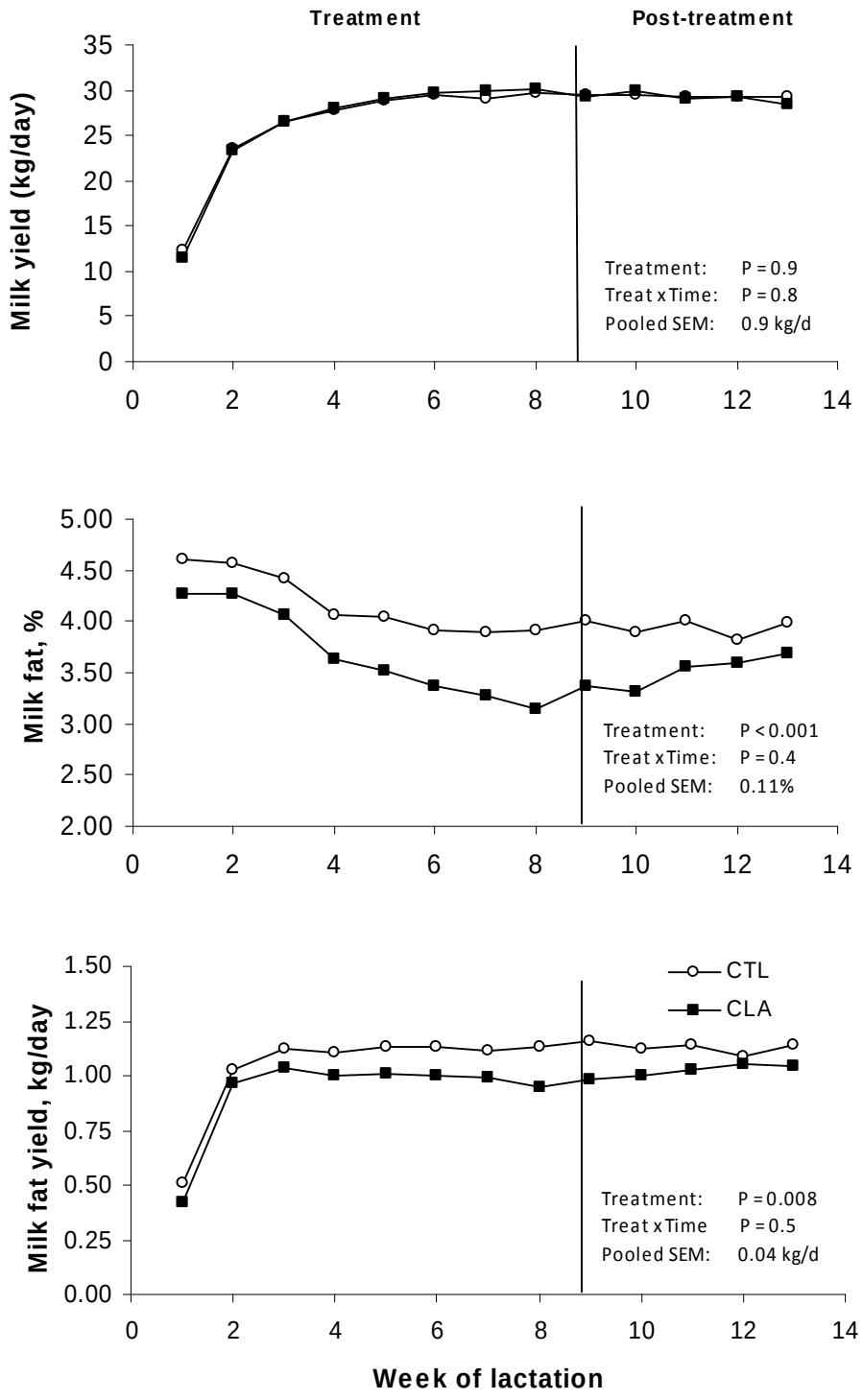
640 [1] CTL = Control; LE-CLA = Lipid Encapsulated Conjugated Linoleic Acid
641

642 Figure 1. Temporal changes in milk yield, milk fat concentration and milk fat yield
643 during the treatment and post-treatment periods. The treatment period lasted from
644 parturition to 60 DIM, and cows were fed either 60 g/day of calcium salts of fatty
645 acids (CTL) or 80 g/day of LE-CLA (LE-CLA). All values are LSM.
646

647 Figure 2. Effect of treatment on dry matter intake, energy balance and body condition
648 loss. The treatment period lasted from parturition to 60 DIM, and cows were fed
649 either 60 g/day of calcium salts of fatty acids (CTL) or 80 g/day of lipid-encapsulated
650 LE-CLA (LE-CLA). All values are LSM.
651

652 Figure 3. Temporal changes in circulating glucose, NEFA and BHBA in cows
653 supplemented with CTL and LE-CLA supplements. The treatment period lasted from
654 parturition to 60 DIM (depicted by the solid black bar), and cows were fed either 60
655 g/day of calcium salts of fatty acids (CTL) or 80 g/day of LE-CLA (LE-CLA).
656 Glucose, NEFA and BHBA values were not normally distributed and were log-
657 transformed prior to analysis to generate a normal distribution.

Figure 4. Plasma insulin concentrations and R-QUICKI values in CTL and LE-CLA supplemented cows. The treatment period lasted from parturition to 60 DIM (depicted by the solid black bar), and cows were fed either 60 g/day of calcium salts of fatty acids (CTL) or 80 g/day of LE-CLA (LE-CLA). Upper panel: Insulin values were log-transformed prior to analysis to generate a normal distribution. Lower panel: R-QUICKI values were generated by taking both the logarithm and reciprocal of glucose×insulin×NEFA. The R-QUICKI values generated were not normally distributed, and were log-transformed prior to analysis.

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685 Figure 1 - Hutchinson

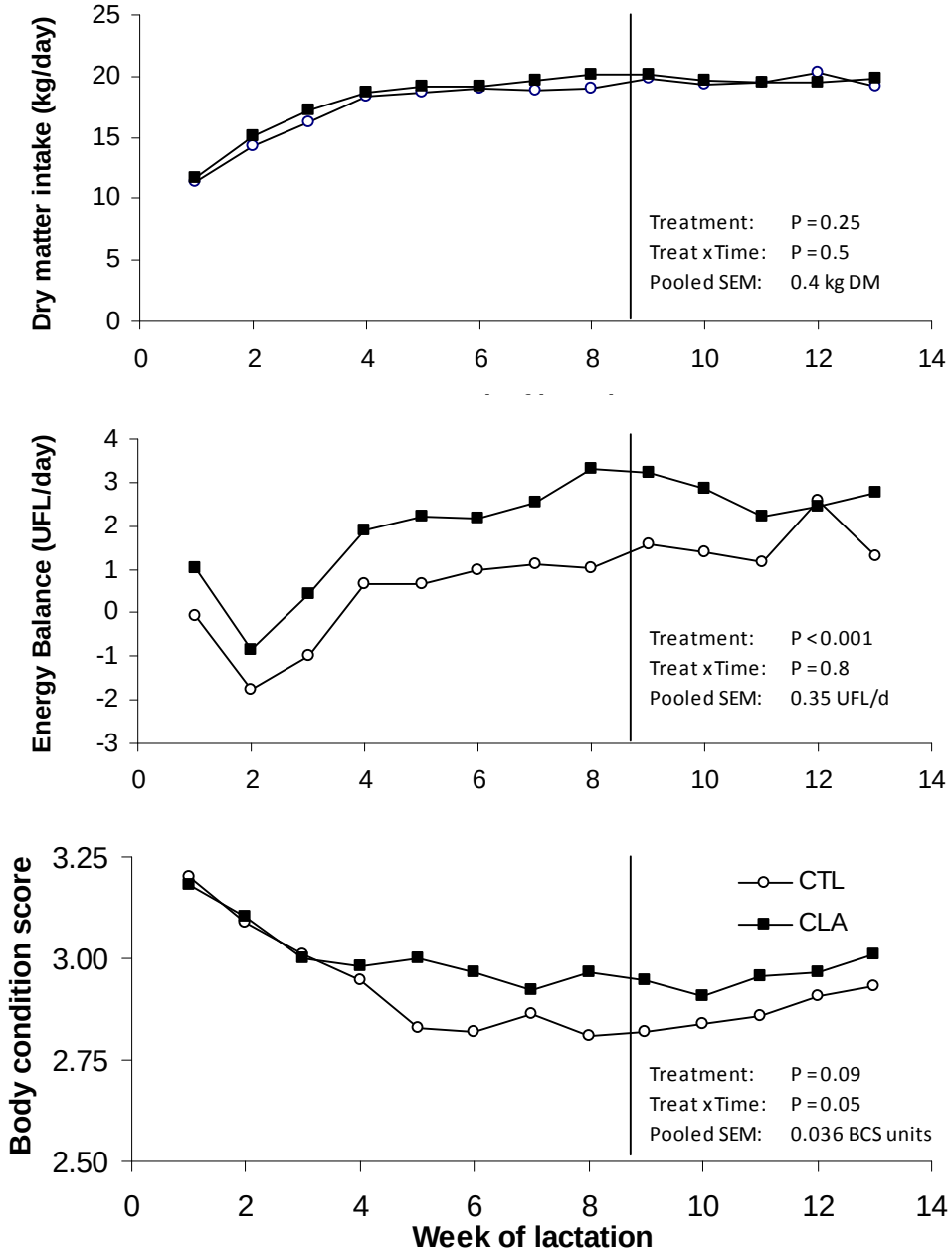


Figure 2 - Hutchinson

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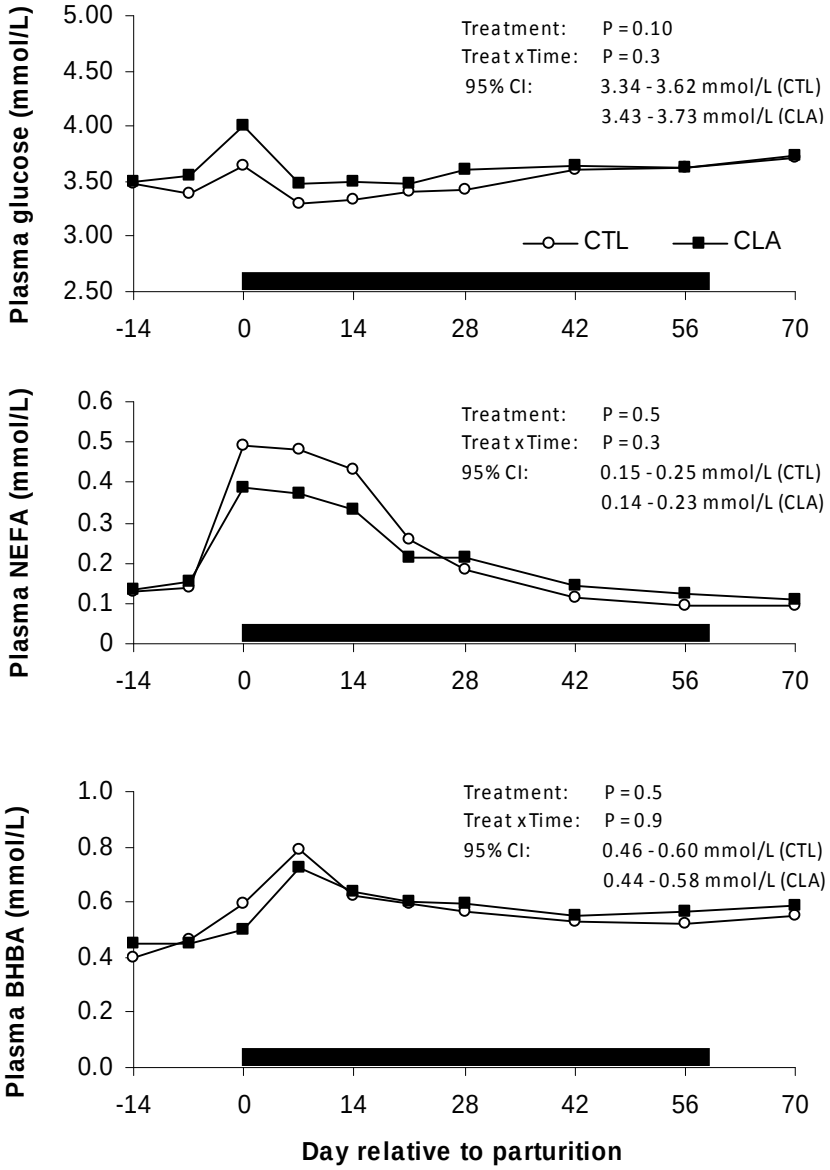


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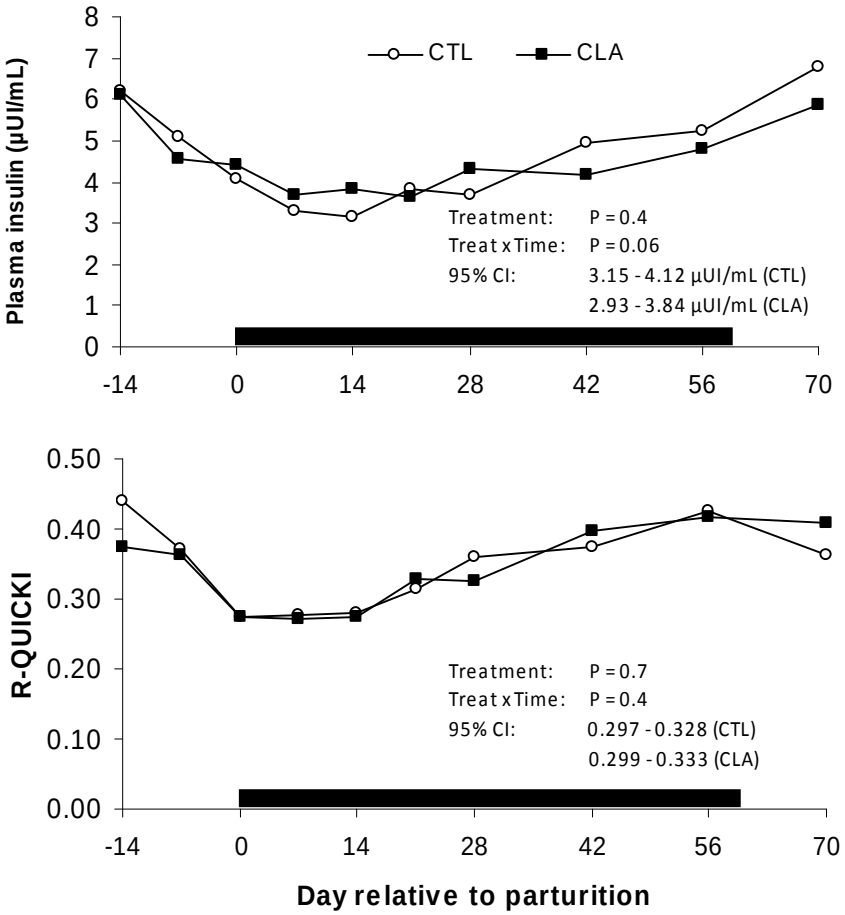


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743 Figure 4 - Hutchinson
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