Interpretive summary: LE-CLA and Reproduction. Hutchinson

Reducing milk energy output in early lactation could be a viable strategy to improve energy status and subsequent fertility in dairy cows, thereby reducing the economic impact of poor reproductive performance. Cows were fed either a conjugated linoleic acid supplement or a control supplement daily for 60 d after calving. Milk production, milk progesterone concentrations, and reproductive performance were monitored. Milk fat production was reduced but milk yield was increased, resulting in no net energy saving effect. Reproductive performance was unaffected by supplementing lactating dairy cows with conjugated linoleic acid.

The effect of strategic supplementation with *trans*-10 *cis*-12 conjugated linoleic acid on the milk production, estrous cycle characteristics, and reproductive performance of lactating dairy cattle

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ABSTRACT

The objective was to determine the effects of a protected (lipid-encapsulated) conjugated linoleic acid (LE-CLA) supplement on milk production, estrous cycle characteristics, and reproductive performance in lactating dairy cows on a pasture-based diet. Spring calving dairy cows (n = 409) on a single pasture-based commercial dairy farm were used in a completely randomized block design. Cows were assigned to 1 of 2 dietary supplements (LE-CLA (Lutrell®, BASF, Germany; n = 203) or no supplement (Control, n = 206)). The LE-CLA cows received 51 g/d of a lipid supplement containing 5 g of both *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA from 0 to 60 d in milk. Milk samples were collected 3 times weekly, and each sample was analyzed for progesterone to determine interval to first ovulation and estrous cycle characteristics. Milk yield and concentrations of fat, protein, and lactose were measured fortnightly. Cows were inseminated following visual observation of estrus. The breeding season commenced on April 8, 2009 and continued for 16 wk. Trans-rectal ultrasonography was carried out at 30 to 36 d and 60 to 66 d post AI to diagnose pregnancy. The LE-CLA treatment resulted in a reduction in milk fat concentration (36.9 g/kg ± 0.06 g/kg vs. 30.7 g/kg ± 0.06 g/kg Control and LE-CLA, respectively) and yield (0.91 kg/d ± 0.02 kg/d vs. 0.84 kg/d ± 0.02 kg/d Control and LE-CLA, respectively); however, milk yield was increased by LE-CLA supplementation (24.7 kg/d ± 0.7 kg/d vs. 27.2 kg/d ± 0.7 kg/d, Control and LE-CLA, respectively), resulting in no overall difference in milk energy output. There was no effect of LE-CLA on any estrous cycle characteristics or measures of reproductive performance. These results suggest that in pasture-based systems of dairy production, where energy intake limits milk production, energy spared by CLA-induced milk fat depression is partitioned towards increasing milk yield rather than towards body reserves.

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

INTRODUCTION

The early postpartum period in dairy cattle is associated with negative energy balance (**NEB**) and mobilization of body reserves, as the energy requirements for maintenance and milk production exceed energy ingested (Bauman and Currie, 1980). Both the duration and severity of this period of NEB have detrimental effects on follicle development, postpartum resumption of ovarian cyclicity, and subsequent oocyte and embryo quality, resulting in reduced pregnancy rates (Beam and Butler, 1999; Diskin et al., 2003; Leroy et al., 2008).

Attempts to alleviate NEB have largely focused on increasing the energy density of the diet. Feeding supplemental fat generally results in an increase in nutrients partitioned towards milk production (Santos et al., 2008), or else DMI is suppressed so that total energy intake is unaffected (Staples et al., 1998).

Improved reproductive performance was reported in some studies where lactating dairy cows were supplemented with polyunsaturated fatty acids. Although results have been inconsistent (Santos et al., 2008), it would appear that reproductive performance may be improved by the specific effects of individual fatty acids, independent of energy status.

Conjugated linoleic acids (**CLA**) are geometric and positional isomers of linoleic acid, and are normally found in the rumen as intermediates in the biohydrogenation of linoleic to stearic acid. *Trans*-10, *cis*-12 CLA was identified as a potent inhibitor of milk fat synthesis (Baumgard et al., 2002), with a dose-dependent response of up to 50% reduction in milk fat synthesis (de Veth et al., 2004). Milk volume and milk protein concentration were not reduced by CLA supplementation, and milk fat concentration quickly returned to control levels on termination of CLA supplementation (Castaneda-Gutierrez et al., 2007). Fat is the most energetically expensive component of milk, representing up to 35% of net energy intake in early lactation (Bauman and Currie, 1980). Feeding supplemental CLA may be a means of reducing milk energy output and ameliorating NEB postpartum, with subsequent

improvements in reproductive performance. Some studies reported no improvement in energy status, with energy spared being partitioned towards increased milk volume (Bernal-Santos et al., 2003), whereas other reports indicated reductions in milk energy output with CLA supplementation (Odens et al., 2007; Hutchinson et al., 2011). A recent meta-analysis of 5 controlled studies in which early-lactation dairy cows had been supplemented with CLA indicated that CLA supplementation reduced interval to first ovulation and time to conception, and increased the probability of cows becoming pregnant (de Veth et al., 2009). The studies included in the meta-analysis utilized a small number of cows (total n = 212), and determining the effects of CLA on reproductive performance was not a primary objective in any of the 5 studies included in the meta-analysis. To date, there has been no herd scale evaluation of the effect of lipid-encapsulated CLA on reproductive performance in lactating dairy cattle. The aim of the present study was to examine the effects of CLA supplementation on milk production and reproductive performance of lactating dairy cows in a pasture-based system of production under commercial conditions.

MATERIALS AND METHODS

Animals, Treatments and Sampling

A total of 409 primiparous and multiparous spring-calving Holstein-Friesian dairy cows on a single pasture-based commercial dairy farm in County Cork, Ireland (52°05'N; 8°16'W) were blocked on the basis of parity (1, 2, and > 2) and calving date, and randomly assigned to receive 1 of 2 dietary treatments from parturition until 60 DIM: protected (lipid-encapsulated) **LE-CLA** (n = 203; Lutrell®, BASF, Ludwigshafen, Germany) or no supplement (Control, n = 206). The mean calving date was 23rd February 2009 (SD = 29 d; range = 4 January 2009 to 26 April 2009). Prior to parturition, all cows were managed in the same manner, housed indoors, and fed a grass silage diet. Following parturition, the cows were managed as a single herd and milked twice daily at 0700 and 1600 h in a 60-unit rotary

parlor with automatic cow identification, automatic concentrate feeding, and electronic milk meters (Dairymaster, Causeway, Kerry, Ireland). Every cow was offered the same basal diet of grazed grass and a concentrate ration fed in the parlor at milking times. In late winter and early spring, when grass growth was limited by weather conditions, they were fed a forage diet based on a 50:50 mix of grass silage and corn silage supplemented with 2 kg/cow per day of soybean meal. The parlor concentrate supplement was fed at a rate of 6 kg/cow per day in late winter, and was gradually reduced to 1 kg/cow per day as grass growth increased. The chemical composition of the feeds offered (Partial Mix Ration, silage mix, grazed grass, and parlor concentrate supplement) is shown in Table 1. The LE-CLA cows were individually fed an additional 51 g/d of lipid supplement from parturition until 60 DIM. The LE-CLA supplement contained a 50:50 mix of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, resulting in a daily intake of 5 g/d of each isomer. The LE-CLA supplement was automatically dispensed to individual cows in granular form using a PowerDos® feeding system (Hanskamp AgroTech BV, Zelhem, The Netherlands) simultaneous with the concentrate allocation. The PowerDos® system delivered the supplement from a hopper via a pneumatic stainless steel dosage mechanism. As this mechanism was able to deliver a maximum of 17 g of LE-CLA in a single dose, the feeding system was programmed to feed a double dose (34 g) at the morning milking and a single dose (17 g) at the evening milking. The feeding system was tested a number of times before the initiation of the study and at fortnightly intervals to ensure that the correct amount of LE-CLA was being provided to the cows. Milk yield was recorded daily at morning and evening milkings using electronic milk meters (Dairymaster). Milk composition (fat, protein, and lactose), was determined on a fortnightly basis from successive morning and evening milk samples by automated infra-red absorption analysis using a Milkoscan 605 (Foss Electric, Hillerød, Denmark).

149 The following equation was used to determine the milk energy output (O' Mara,
150 1997), using unité fourragère lait (**UFL**) as the unit of net energy, which is equivalent to 1 kg
151 of standard air-dried barley.
152 Energy requirement for milk (UFL/kg of milk) = 0.0054 FC + 0.0031 PC + 0.0028
153 LC - 0.015;
154 where FC = fat concentration (%), PC = protein concentration (%), and LC = lactose
155 concentration (%).
156 A total of 20 cows (9 Control, 11 CLA), were removed from the experiment due to
157 illnesses and metabolic problems unrelated to dietary treatment.
158 ***Milk progesterone sampling and analysis***
159 Milk samples from all cows enrolled in the study were collected at the morning
160 milking 3 times weekly (Monday, Wednesday, and Friday); a preservative was added to each
161 sample (Lactab Mark III, Thomson and Capper Ltd., Cheshire, UK) and the samples were
162 stored at 4 °C until analysis. Milk progesterone (**P4**) concentrations were measured in
163 representative samples from every cow on each sampling date using a competitive ELISA
164 test (Ridgeway Science, Gloucester, UK), based on published methods (Sauer et al., 1986).
165 The inter- and intra-assay coefficients of variation were 14.5 % and 9.1 %, respectively, and
166 the sensitivity of the assay was 0.5 ng/mL (Sauer et al., 1986).
167 ***Interval to first ovulation***
168 A period of luteal activity was defined as the occurrence of 2 or more consecutive
169 milk P4 concentrations ≥ 3 ng/mL (Darwash et al., 1997). The interval to first ovulation
170 (**IOV1**) was defined as the first occurrence of luteal activity postpartum, or the first day on
171 which milk P4 concentrations ≥ 3 ng/mL were observed, and meeting the above criteria.
172 ***Abnormal estrous activity***

Abnormal estrous activity was identified based on previously published criteria (Royal et al., 2000). Prolonged anovulation postpartum, delayed ovulation type I (**DOV I**), was defined as milk P4 concentrations < 3 ng/mL for ≥ 45 DIM. Prolonged inter-luteal interval, delayed ovulation type II (**DOV II**), was defined as milk P4 concentrations < 3 ng/mL for ≥ 12 d after the first occurrence of luteal activity postpartum. Delayed luteolysis during the first estrous cycle postpartum, persistent corpus luteum type I (**PCL I**), was defined as milk P4 concentrations ≥ 3 ng/mL for ≥ 19 d during the first postpartum estrous cycle. Delayed luteolysis during subsequent estrous cycles before AI, persistent corpus luteum type II (**PCL II**), was defined as milk P4 concentrations ≥ 3 ng/mL for ≥ 19 d during subsequent postpartum estrous cycles.

Characteristics of the estrous cycle outlined above were determined before the first postpartum AI. In total, results were available from 306, 371, 223, 250, and 146 cows for IOV1, DOVI, DOVII, PCLI, and PCLII, respectively.

Fatty acid analysis

Milk samples were collected from 15 cows on each treatment at 30 and 60 DIM, and the samples were analyzed for milk fatty acid composition. The samples were analyzed by gas liquid chromatography according to the method developed by (Collomb et al., 2000) following extraction and methylation according to ISO standards 14156:2001 (ISO, 2001) and 15884:2002 (ISO, 2002). Samples of the feed offered were collected at these time points and fatty acid content was determined by gas liquid chromatography as described by Childs et al (2008) following lipid extraction using a chloroform, methanol and water mixture (Folch et al., 1957), and methylation using $NaOCH_3$, methanol, and BF_3 (Park and Goins, 1994). The fatty acid composition of the feeds offered is shown in Table 2.

Breeding and reproductive performance

Breeding commenced on April 8, 2009, and continued for 16 wk. Artificial insemination was carried out by a single experienced technician, and took place after morning and evening milkings. Cows were AI 12 h after first showing signs of estrus. Tail paint and MooMonitor activity collars (Dairymaster) were used to aid heat detection. Body condition score was assessed (Edmonson et al., 1989) on 4 fixed calendar dates during the breeding season. The BCS assessment commenced on the 5 April 2009 and took place on fixed calendar dates at intervals of approximately 4 wk thereafter. As a result, some of the LE-CLA cows were still receiving the LE-CLA supplement for the first 2 BCS assessment dates, whereas others had finished the LE-CLA supplementation period before the first BCS assessment date of April 5, 2009. Trans-rectal ultrasonography was carried out at 30 to 36 d and 60 to 66 d post AI using a 5.0-MHz transrectal transducer (Aloka SSD-500; Aloka Ltd., Tokyo, Japan) to diagnose pregnancy. Visualization of a fluid-filled horn and a viable embryo were used for positive identification of pregnancy. Three-week submission rate was defined as the proportion of cows inseminated within the first 3 wk of the breeding season. Six-week in calf rate was defined as the proportion of cows pregnant within the first 6 wk of the breeding season. Overall pregnancy rate was defined as the proportion of cows pregnant at the final herd scan on the 2 December 2009. Cows that underwent embryo loss were defined as cows that were diagnosed as pregnant at 30 to 36 d post-AI, but were then diagnosed as non-pregnant at 60 to 66 d post-AI.

Statistical analysis

All statistical analysis was carried out using SAS (SAS System Inc., Cary, NC). Milk production, milk composition, and BCS data were analyzed using the MIXED procedure of SAS 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 model fit statistic. A first order

autoregressive covariance structure was selected. Treatment, treatment week, and their interaction were included as fixed effects, and block was included as a random effect. 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. Data for IOV1, the interval from calving to first AI, and interval from calving to conception were evaluated by the LIFETEST procedure of SAS using Kaplan-Meier analysis to investigate the effect of treatment on the number of days from calving to commencement of luteal activity, first AI, and conception. The IOV1 was right-censored at 60 d, calving to service interval was right-censored at the last date of AI use (15 June), and calving to conception interval was right-censored at the last date of the breeding period (25 September). All of the binary reproductive performance variables were analyzed using the FREQ procedure of SAS with the Chi-squared test.

RESULTS

Milk production and BCS

The milk production results are summarized in Table 3 and Figure 1. Milk fat concentration was reduced by LE-CLA treatment ($P < 0.001$), but the treatment by time interaction effect was not significant. During the supplementation period, milk fat yield was reduced ($P = 0.03$) by up to 8%, milk yield was increased ($P = 0.003$), and milk protein concentration was reduced ($P < 0.001$). Milk solids yield (fat plus protein) was not affected. Milk fat concentration and yield in LE-CLA supplemented cows began to return towards levels similar to control cows after the end of the supplementation period (Figure 1). Supplementing cows with LE-CLA tended to increase BCS ($P = 0.09$, Figure 2).

Estrous cycle characteristics

Estrous cycle characteristics and IOV1 results are presented in Table 4. There was no effect of LE-CLA supplementation on IOV1 (40.2 ± 1.05 d vs. 40.3 ± 1.19 d, Control and

LE-CLA respectively, Log Rank Probability of Chi-Square test = 0.87). The proportion of cows that continued to be anestrous at 60 DIM was 0.23 and 0.25 for Control and CLA, respectively. Incidence of DOV I (35.1% vs. 36.1%) and DOV II (16.7% vs. 14.6%) were not affected by LE-CLA supplementation (both $P > 0.6$). The LE-CLA supplementation had no effect on the incidence of PCL I (8.9% vs. 10.3%) or PCL II (8.9% vs. 7.5%, both $P > 0.6$).

Reproductive performance

Reproductive performance data are summarized in Table 5. There was no effect of LE-CLA supplementation on the interval from calving to first insemination (71.8 ± 1.84 d vs. 70.9 ± 1.79 d, Control and LE-CLA, respectively, Log Rank Probability of Chi-Square test = 0.5), or the interval from calving to conception (123.7 ± 4.68 d vs. 130.4 ± 4.66 d, Control and LE-CLA, respectively, Log Rank Probability of Chi-Square test = 0.2). There was no effect of LE-CLA supplementation on conception rate to first (35.1 vs. 37.0; Control and LE-CLA, respectively) or second service (38.5% vs. 29.9%, both $P > 0.2$). There was also no effect of CLA supplementation on 3 week submission rate (54.8% vs. 58.0%, $P = 0.5$), embryo loss to first service (15.2% vs. 17.9%, $P = 0.7$), 6 week in-calf rate (43.6% vs. 37.0%, $P = 0.2$), or overall pregnancy rate (80.7% vs. 76.0%, $P = 0.3$).

Milk fatty acid analysis

CLA supplementation reduced (all $P < 0.01$) the proportion of most short and medium chain fatty acids in milk fat compared to control animals (Table 6), with the exception of C4:0, C14:0 and C15:0 which were not affected (all $P > 0.3$). The proportion of C16:0 in milk fat was not affected by CLA supplementation ($P > 0.1$). The LE-CLA supplementation tended to increase ($P = 0.07$) the proportion of C18:0 in milk fat, and increased the proportion of *cis*-9 C18:1 and *cis*-9, *trans*-11 CLA in milk fat (both $P < 0.04$). There was no effect of CLA supplementation on any other long chain fatty acids measured (all $P > 0.08$). Overall, this resulted in a decrease in the proportion of de novo synthesized (< C16:0) fatty

acids (P = 0.03), and an increase in the proportion of preformed (> C17:0) fatty acids in the milk fat of cows fed the LE-CLA supplement.

DISCUSSION

The feeding and management systems utilized in this study enabled accurate and reliable supplementation with LE-CLA to individual cows on a large herd scale, the first time this has been achieved. We have demonstrated that LE-CLA supplementation during the first 60 DIM can be used as a management tool to temporarily reduce milk fat synthesis in pasture-based dairy cows. Despite observing a trend towards an improvement in BCS in cows supplemented with LE-CLA, the lack of an effect of LE-CLA on milk energy output suggests that most energy spared by reducing milk fat synthesis was partitioned towards increasing milk production. There was no effect of LE-CLA supplementation on any measure of reproductive performance or estrous cycle characteristics.

Fat is an economically important component of milk to dairy farmers. Extreme milk fat depression (MFD) may be undesirable, especially in regions where milk is primarily used for manufacturing purposes. Results from a previous study (Hutchinson et al., 2011) provided the justification for the level and duration of LE-CLA supplementation used in the present study to induce sufficient MFD to potentially improve energy status, but not markedly decrease income from milk.

The CLA dose fed in the present study (5 g/d *trans*-10, *cis*-12 CLA) is similar to that fed by Hutchinson et al. (2011), although the supplement was fed in pelleted form, whereas in the current study the granular supplement was fed directly to the cows, avoiding any potential degradation of the supplement during the pelleting process. In the current study a reduction in milk fat concentration in LE-CLA cows occurred within a week after the initiation of treatment. There is no evidence of any adaptation to the treatment, as the data in Figure 1 show that maximal milk fat depression occurred at wk 8 postpartum, at the end of the

297 treatment period. We observed a maximum depression in milk fat concentration of 18.8%,
298 greater than the 15.7% observed by (Hutchinson et al., 2011), who fed the same supplement
299 but at a greater dose of 6.9 g/d of *trans*-10, *cis*-12 LE-CLA. The greater reduction in milk fat
300 concentration in the present study may suggest an improved efficacy of the supplement, most
301 likely attributable to avoiding any potential degradation during the pelleting process, as noted
302 by Hutchinson et al., (2011).

303 The increase in milk yield in the current study supports the work of Bernal-Santos et
304 al. (2003) and Mackle et al. (2003), but differs from the findings of Hutchinson et al. (2011),
305 and Castaneda-Gutierrez et al. (2005). Moore et al. (2004) hypothesized that during early
306 lactation the extra energy afforded by a reduction in milk fat synthesis may be partitioned
307 towards protein synthesis and milk production. We observed an increase in milk production,
308 though there was a small, but statistically significant, reduction in milk protein concentration.
309 Because of the increased milk yield, however, milk protein yield was not affected. There was
310 also a reduction in milk lactose concentration with LE-CLA supplementation, but milk
311 lactose yield was increased due to greater milk volume. To our knowledge, the current study
312 is the first to report a reduction in milk lactose concentration with CLA supplementation.
313 Overall, although milk constituent concentrations were reduced, the increase in milk
314 synthesis negated these effects and resulted in no differences in milk solids yields.

315 In the current study LE-CLA supplementation caused a reduction in the secretion of
316 all fatty acids, but those of de novo origin were reduced to a greater extent. The LE-CLA
317 reduced the proportion of *de novo* fatty acids and increased the proportion of preformed fatty
318 acids in milk fat, results that support previous reports (Kay et al., 2007; Moore et al., 2004;
319 Perfield et al., 2002). *Trans*-10, *cis*-12 CLA reduces milk fat synthesis through coordinated
320 decreases in expression of genes encoding key enzymes involved in the uptake and transport

of preformed fatty acids, in addition to enzymes involved in the desaturation of fatty acids, formation of triglycerides, and de novo fatty acid synthesis (Baumgard et al., 2002).

Although the milk fat depressing effects of CLA are generally accepted, the subsequent effect on energy status is more equivocal. Some studies reported improvements in energy status with CLA supplementation (Hutchinson et al., 2011; Odens et al., 2007); however, absence of an effect of CLA on energy status was also reported in a number of studies (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005). In studies where CLA did not improve energy status, it was generally hypothesized that any energy saved from the reduction in milk fat synthesis was partitioned towards greater milk production, such that milk energy output remained unchanged (Bernal-Santos et al., 2003). The present study differs from previous reports in that milk energy output was unaffected by LE-CLA supplementation, but a trend towards improved BCS in LE-CLA treated cows was also observed. Although we detected a statistical trend towards improved BCS in LE-CLA supplemented cows, the degree to which BCS was improved is marginal (less than 0.05 BCS units), and the ability to detect an improvement in BCS was most likely attributed to the large number of cows on the study. Due to the on-farm nature of the current study, it was not possible to measure DMI. In previous studies where DMI has been measured, CLA had no effect on DMI in cows consuming a TMR (Bernal-Santos et al., 2003; Moore et al., 2004) or pasture (Kay et al., 2007; Kay et al., 2006) diet.

Mackle et al. (2003) suggested that energy spared from a reduction in milk fat synthesis is likely to have a greater positive impact on milk production in pasture-fed cows than cows fed a TMR diet that could more closely meet energy requirements. The current study, along with data from Kay et al. (2006), Mackle et al. (2003), and Medeiros et al. (2010) support this hypothesis. The work of Kay et al. (2007) is the only study in cows receiving a pasture diet in which CLA-induced MFD resulted in decreased milk energy

output, but was not accompanied by an increase in milk yield. In that study much greater levels of MFD were achieved (> 40%) due to abomasal infusion of the CLA, which avoided rumen biohydrogenation of the supplement. Positive milk yield responses were not observed at more severe (> 35%) levels of MFD (Kay et al., 2006). It is reasonable to conclude that in pasture-based systems of dairy production, where milk production is limited by energy intake, the extra energy spared by CLA-induced MFD is partitioned towards milk production rather than body reserves. It seems likely that overall energy status is only improved in situations where the cow is already producing at full potential.

There was no effect of LE-CLA on any of the reproduction variables or estrous cycle characteristics measured. No previous work in which dairy cows were supplemented with CLA investigated the effects on estrous cycle characteristics, although there is a body of work from which we can draw general comparisons. The overall incidence of both DOV I (35.1%) and DOV II (15.7%) in the current study is greater than observed by Opsomer et al. (1998) (20.5% and 3%) and Royal et al. (2000) (12.9% and 10.6%). The high incidence of delayed ovulation observed in the present study is indicative of a widespread fertility problem in the herd, as cows with extended postpartum anestrus intervals had lower submission, conception, and pregnancy rates than cycling animals (McDougall et al., 2001). This is reflected in the sub-optimal fertility performance of the herd, with conception rates to first service in the current study (35.1 to 37.0%) less than reported in pasture-based systems (Horan et al., 2004: 47 to 56%; Buckley et al., 2003: 49%). Similarly, the embryo loss rates of 15.2 to 17.9% were greater than those reported by Silke et al. (2002) (6.1 to 7.2%) and Horan et al. (2004) (7.5%), from comparable studies in Irish pasture-based dairy herds. Figures of 54 to 58% for 3-wk submission rate and 37 to 44% for 6-wk in-calf rate in the current study are less than the target rates for seasonal-calving pasture based systems of > 80% for 3-wk submission rate and > 68% for 6-wk in-calf rate (McDougall, 2006).

There are a wide range of latent factors that act to negatively impact reproductive performance in dairy cows on commercial dairy farms. These include infectious disease status, macro and trace mineral nutrition status, and a variety of other stressors. It is possible that LE-CLA supplementation has no beneficial effect on reproductive performance, as the results of this study suggest. It is also possible, however, that one or more factors may have been present on the farm that acted to antagonize cow fertility, and this overrode any potential beneficial effect of LE-CLA.

CONCLUSIONS

Supplementation with LE-CLA induced milk fat depression in early lactation, pasture fed dairy cows. Due to increased milk yield in LE-CLA supplemented cows, there was no effect on milk energy output. There was no effect of LE-CLA supplementation on any estrous cycle characteristic or measure of reproductive performance. In grass-based systems of dairy production, where milk production is limited by energy intake, any energy spared by a reduction in milk fat synthesis is partitioned towards increased milk production rather than improving the energy status and subsequent reproductive performance of the cow.

ACKNOWLEDGMENTS

The authors would like to thank Mr. J. Crimmins (Curraghdermot Farm, Castlelyons), Mr. P. Crimmins (Curraghdermot Farm, Castlelyons) and the Curraghdermot Farm staff for management and care of the animals. The technical assistance of Mr. B. Curtin (Teagasc, Moorepark) is also appreciated. National Development Plan funding is gratefully acknowledged.

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Table 1. Chemical composition of the feeds offered to dairy cows on pasture.

Nutrient Composition (g/kg DM)	Grass	PMR[1]	Concentrate
OM digestibility	818.3	706.0	-
CP	256.4	120.1	249.2
NDF	487.6	480.0	-
ADF	253.8	274.7	-
Crude fiber	-	-	109.2
Oil	-	-	38.4
Ash	90.6	64.8	80.7

[1]Partial Mix Ration

Table 2. Fatty acid composition (g/100 g of total fatty acids) of the feeds offered to dairy cows on pasture

	% of total fatty acids

Fatty acid	Grass	PMR[1]	Concentrate	LE-CLA[2]
12:0	1.87	1.16	0.57	0.34
12:1	1.71	1.52	0.03	--
13:0	1.09	1.08	--	0.34
13:1 *cis*-12	1.28	0.68	--	--
14:0	1.54	3.88	0.93	1.11
trans 14:1	--	0.12	--	--
15:0	--	--	0.42	0.13
15:1 *trans*-10	2.33	1.79	0.16	--
15:1 *cis*-10	3.86	2.69	--	--
16:0	20.32	26.68	32.29	13.96
cis 16:1	2.31	1.16	0.63	0.07
trans 16:1	--	1.06	--	--
17:0	--	0.23	0.17	0.21
17:1 *cis*-10	--	0.29	--	0.04
18:0	1.03	2.24	1.70	33.28
18:1 *cis*-9	1.62	5.51	13.23	12.07
18:1 *trans*-9	--	0.52	1.50	0.23
18:2 *cis*-9, *cis*-12	5.58	15.15	36.32	1.01
18:2 *cis*-9, *trans*-11 CLA[3]	--	--	--	12.42
18:2 *trans*-10, *cis*-12 CLA	--	--	--	13.72
All *trans* 18:2	0.57	0.80	0.17	2.60
18:3 *cis*-6, *cis*-9, *cis*-12	--	0.11	--	--
18:3 *cis*-9, *cis*-12, *cis-15*	41.76	18.05	3.20	0.02
20:0	0.07	0.44	0.44	1.10

20:1 *cis*-11	--	--	0.45	0.09
20:2 *cis*-11, *cis*-14	--	--	--	0.03
22:0	1.10	2.01	0.74	2.69
22:1	1.85	0.69	0.16	--
22:4	1.41	2.74	1.44	1.23
Total	91.36	90.60	94.53	96.69

[1]Partial Mix Ration

[2]Lipid encapsulated conjugated linoleic acid

[3]Conjugated linoleic acid

528 **Table 3.** Milk production and composition of cows on lipid-encapsulated conjugated linoleic

529 acid (LE-CLA) and control treatments

				P-value	
	Control	LE-CLA[1]	SEM	Trt	Trt x Time
Milk yield (kg/d)	24.7	27.2	0.7	0.003	0.7
Milk fat (g/kg)	36.9	30.7	0.6	<0.001	0.2
Milk fat yield (kg/d)	0.91	0.84	0.02	0.031	0.7
Milk protein (g/kg)	32.8	31.2	0.3	<0.001	0.9
Milk protein yield (kg/d)	0.81	0.85	0.02	0.11	0.9
Milk lactose (g/kg)	47.5	46.4	0.3	0.028	0.9
Milk lactose yield (kg/d)	1.18	1.28	0.03	0.012	0.9
Milk solids yield (kg/d)[2]	1.72	1.69	0.05	0.6	0.9
FCM yield (kg/d) [3]	25.39	25.33	0.66	0.9	1.0
Milk energy output (UFL[4]/d)[5]	10.32	10.32	0.27	1.0	1.0
SCS[6]	101.6 (73.2 - 141.1)	96.2 (69.3 -133.8)	-	0.8	0.3

530 [1]Lipid encapsulated conjugated linoleic acid

531 [2]Milk solids yield = milk fat yield (kg/d) + milk protein yield (kg/d)

532 [3]3.5% FCM yield = 0.4318 * milk yield (kg/d) + 16.23 * milk fat yield (kg/d)

533 [4]UFL = unité fourragère lait; unit of net energy, equivalent to 1 kg of standard air-dried

534 barley

535 [5]Milk energy output = 0.054 * fat concentration (%) + 0.031 * protein concentration (%) +

536 0.028 * lactose concentration (%) - 0.015.

[6]SCS is calculated by taking the natural logarithm of SCC values. Values are back-transformed least square means followed by 95% confidence limits in parenthesis.

552 **Table 4.** Estrous cycle characteristics of cows on lipid-encapsulated conjugated linoleic acid
553 (LE-CLA) and control treatments

	Control	LE-CLA[1]	P-value
Interval to first ovulation (d)[2]	40.2 (± 1.05)	40.3 (± 1.19)	0.9
Incidence of DOV I (%)[3]	35.1 (67/191)	36.1 (65/180)	0.8
Incidence of DOV II (%)[4]	16.7 (20/120)	14.6 (15/103)	0.7
Incidence of PCL I (%)[5]	8.9 (11/124)	10.3 (13/126)	0.7
Incidence of PCL II (%)[6]	8.9 (7/79)	7.5 (5/67)	0.8

554 [1] Lipid encapsulated conjugated linoleic acid

555 [2]Interval to first ovulation data are mean values followed by the standard error of the mean in parenthesis.

556 [3]DOV I (Delayed ovulation type I) = milk P4 < 3 ng/mL for ≥ 45 days post partum

557 [4]DOV II (Delayed ovulation type II) = milk P4 < 3 ng/mL for ≥ 12 days after the first occurrence of luteal
558 activity

559 [5]PCL I (Persistent corpus luteum type I) = milk P4 ≥ 3 ng/mL for ≥ 19 days during the first post-partum oestrus
560 cycle

561 [6]PCL II (Persistent corpus luteum type II) = milk P4 ≥ 3 ng/mL for ≥ 19 days during subsequent post-partum
562 oestrus cycles

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568 **Table 5.** Reproductive performance of cows on lipid-encapsulated conjugated linoleic acid
569 (LE-CLA) and control treatments

	Control	LE-CLA[1]	P-value
Calving to first service interval (d)[2]	71.8 (± 1.79)	70.9 (± 1.84)	0.5
Calving to conception interval (d)[3]	123.7 (± 4.68)	130.4 (± 4.66)	0.2
3 week submission rate (%)[4]	54.8 (103/188)	58.0 (105/181)	0.5
Conception rate to first service (%)	35.1 (66/188)	37.0 (67/181)	0.7
Conception rate to second service (%)	38.5 (37/96)	29.9 (26/87)	0.2
Embryo loss to first service (%)[5]	15.2 (10/66)	17.9 (12/67)	0.7
6 week in-calf rate (%)[6]	43.6 (82/188)	37.0 (67/181)	0.2
Overall pregnancy rate (%)[7]	80.7 (159/197)	76.0 (146/192)	0.3

570 [1]Lipid encapsulated conjugated linoleic acid
571 [2]Calving to first service interval data are mean values followed by the standard error of the
572 mean in parenthesis.
573 [3]Calving to conception interval data are mean values followed by the standard error of the
574 mean in parenthesis
575 [4]3 week submission rate = proportion of cows inseminated in the first 3 weeks of the
576 breeding season.
577 [5]Cows that underwent embryo loss were defined as cows that were scanned as pregnant at the
578 30 to 36 d post insemination scan, but were then scanned as non-pregnant at the 60 to 66 d
579 post insemination scan.
580 [6]6 week in-calf rate = proportion of cows pregnant in the first 6 weeks of the breeding season
581 [7]Overall pregnancy rate = proportion of cows pregnant at the final herd scan

582 **Table 6.** Milk fatty acid composition (g/100 g total fatty acids) of cows on lipid-encapsulated
583 conjugated linoleic acid (LE-CLA) and control treatments

	Control	LE-CLA[1]	SEM	*P* value
4:0	2.71	2.67	0.073	0.74
6:0	1.86	1.67	0.050	0.004
8:0	1.22	1.04	0.043	0.001
10:0	2.83	2.41	0.131	0.002
10:1	0.27	0.22	0.020	0.008
12:0	3.38	2.97	0.169	0.008
12:1 *cis* and 13:0	0.31	0.27	0.014	0.009
14:0	10.97	10.69	0.398	0.43
15:0	1.60	1.52	0.038	0.129
16:0	25.00	24.03	0.728	0.132
16:1 *cis*-9	1.79	1.78	0.097	0.822
17:0	1.15	1.11	0.022	0.114
18:0	10.98	11.74	0.632	0.07
18:1 *cis*-9	21.58	23.18	0.739	0.032
Other 18:1	7.78	7.71	0.257	0.857
18:2 *cis*-9, *cis*-12	1.28	1.35	0.079	0.312
Other 18:2	1.47	1.56	0.073	0.409
18:2 *cis*-9, *trans*-11 CLA	1.59	1.92	0.104	0.023
18:3 *cis*-9, *cis*-12, *cis*-15	0.67	0.75	0.050	0.116
20:0	0.10	0.11	0.007	0.085
20:5 EPA	0.09	0.09	0.005	0.947
22:0	0.17	0.10	0.053	0.363

22:5 DPA	0.11	0.11	0.008	0.452
Fatty acid origin				
De novo[2]	25.17	23.48	0.820	0.03
16:0 and 16:1	26.81	25.81	0.796	0.145
Preformed[3]	47.13	49.88	1.514	0.026

584 [1]Lipid encapsulated conjugated linoleic acid

585 [2]Milk fatty acids synthesized in the mammary gland of chain length C4 - C15

586 [3]Milk fatty acids derived from the uptake of circulating fatty acids, of chain length greater

587 than C16

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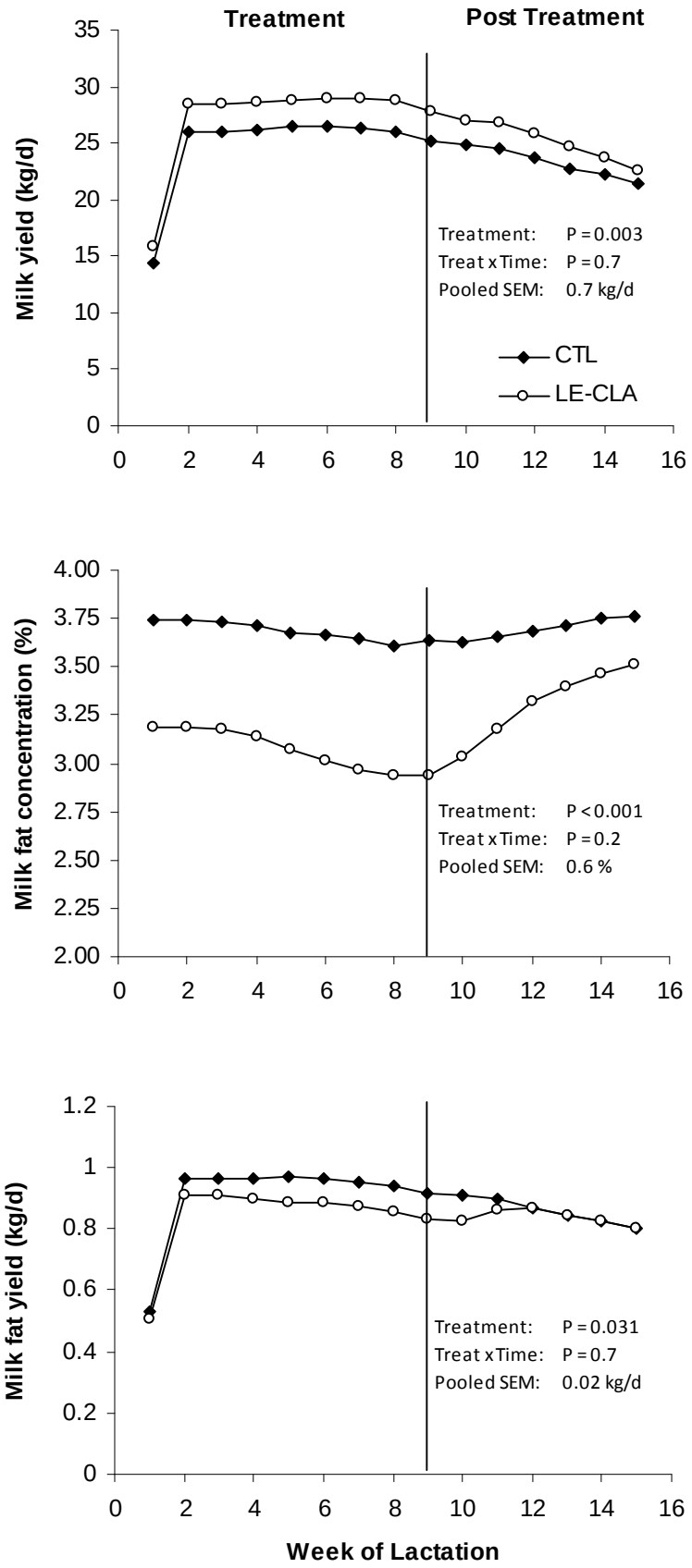

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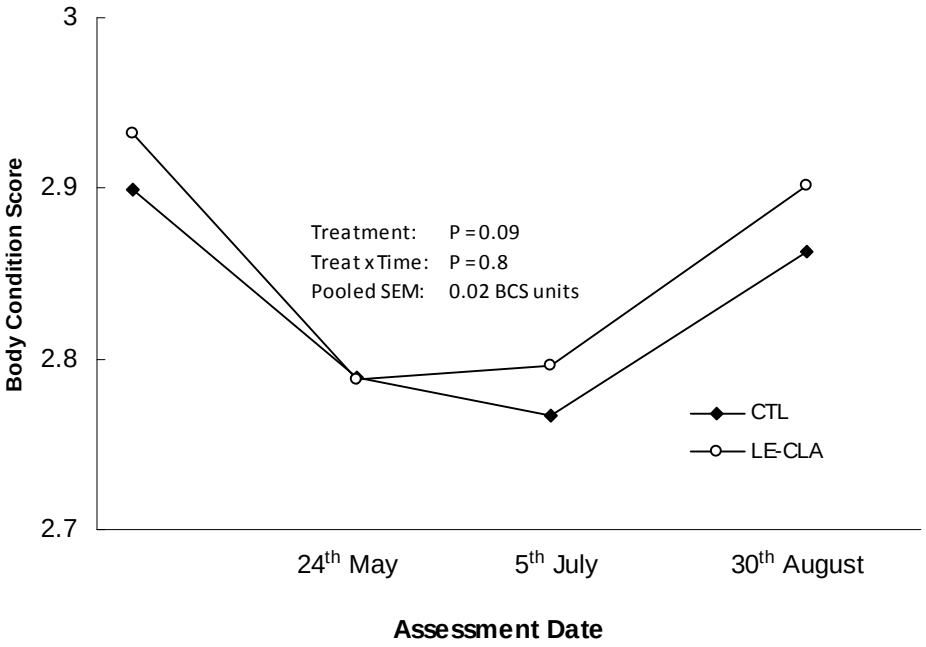
590 Figure 1 - Hutchinson



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592 Figure 2 - Hutchinson

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Figure 1. Temporal changes in milk yield, milk fat concentration, and milk fat yield during the treatment and post-treatment periods. The treatment period lasted from parturition to 60 DIM, and cows were fed either no supplement (CTL), or 51 g/d of lipid-encapsulated conjugated linoleic acid (LE-CLA). The LE-CLA supplement provided 5 g/d of both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. All values are LSM.

Figure 2. Effect of treatment on BCS. Body condition score was assessed on fixed calendar dates, just prior to the start of mating, followed by measurements approximately 6 wk apart. The treatment period lasted from parturition to 60 DIM, and cows were fed either no supplement (CTL), or 51 g/d of lipid-encapsulated conjugated linoleic acid (LE-CLA). The LE-CLA supplement provided 5 g/d of both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. All values are LSM