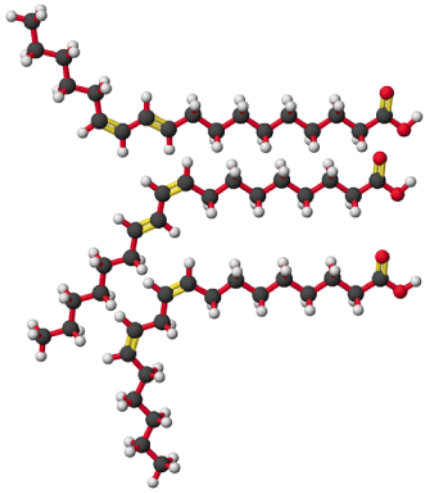
**Project number:** 5890
**Funding source:** Teagasc
**Date:** August 2015
**Project dates:** Sept 2008 - Sept 2013

# Micronutrient effects on dairy cow fertility



Key external stakeholders:
Dairy farmers, nutritionists, feed industry

Practical implications for stakeholders: Studies were undertaken to examine the effects of different fat supplements on the reproductive system of dairy cows.

- Assessed the impact of fat supplements containing conjugated linoleic acid on milk production, reproductive physiology and dairy cow fertility
- Determined the effect of providing different forms of omega-3 fatty acid supplements on reproductive function
- Assessed the potential benefit of supplementing cows with a fat supplement containing saturated fatty acids on progesterone clearance rate

## Main results:

- Supplementing cows with a fat supplement containing trans-10, cis-12 conjugated linoleic acid in early lactation reduced milk fat production and improved energy balance, but did not improve dairy cow fertility performance.
- Supplementing cows with fat supplements containing different sources of omega-3 fatty acids did not improve indicators of fertility.
- In situations of inadequate pasture availability, providing a standard saturated fat supplement tended to increase the progesterone half-life and reduce metabolic clearance rate.

## Opportunity / Benefit:

There is no beneficial effect on fertility of supplementing cows on pasture-based systems with fat supplements containing conjugated linoleic acid. As pasture-based diets are inherently high in the omega-3 fatty acid α-linoleic acid, providing additional supplemental omega-3 fatty acids provided no benefit in the studies conducted.

## Collaborating Institutions:

UCD



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## 1. Project background:

Dairy cow diets are supplemented with fat primarily to increase the energy density of the diet to enhance milk production, growth and reproduction. It was initially thought that feeding energy dense fat supplements in early lactation, when the dairy cow is experiencing a period of negative energy balance, would improve the energy status of the animal and consequently improve reproductive performance. When fat is fed in early lactation, however, cows either consume less feed or increase milk production and energy status is seldom altered. It has also been suggested that any benefits of feeding fat may be independent of energy status, and may instead be due to specific effects on the pituitary gland, ovaries and uterus, mediated by the fatty acid composition of the fat source. Potential improvements in dairy cow fertility with supplemental fat have generally been associated with increased dominant follicle diameter, improved oocyte and embryo quality, greater progesterone (P4) concentrations, and modulation of prostaglandin (PG) synthesis, collectively resulting in increased likelihood of conception.

Trans-10, cis-12 conjugated linoleic acid (CLA), an n-6 polyunsaturated fatty acid (PUFA), is a potent inhibitor of milk fat synthesis, and has recently been demonstrated to decrease milk energy output and improve energy balance. Numerous studies have shown that improvements in energy balance have a beneficial effect on reproductive performance. Other potential mechanisms by which CLA might improve reproductive performance are yet to be fully elucidated.

Flaxseed oil is high in the essential n-3 PUFA α-linolenic acid, and has been shown to increase the size of the dominant follicle and reduce pregnancy losses. Fish oil contains substantial amounts of the long chain n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Recent studies have highlighted the potential to manipulate PG synthesis by adding n-3 PUFA supplements to the diet. Increasing the proportion of n-3 PUFA in the diet can result in increased synthesis of the less biologically active 3-series PGs at the expense of PGF2α. Studies conducted in vitro, and in vivo using beef heifers demonstrated that n-3 PUFA supplementation can alter endometrial expression of genes regulating PGF2α synthesis, potentially leading to a reduction in uterine PGF2α production. Inhibition of uterine PGF2α secretion may delay the regression of the corpus luteum (CL), and hence improve embryo survival.

Saturated fat supplements are primarily used as an energy source, and generally do not elicit any of the specific physiological effects that are stimulated by omega-3 and omega-6 fatty acids. There is some evidence that saturated fat supplementation can reduce the metabolic clearance rate of progesterone. As greater blood progesterone concentrations are generally considered favourable, supplementing cows with saturated fat may have favourable effects for cow fertility.

## 2. Questions addressed by the project:

- Question 1: Does supplementation with CLA during early lactation improve energy balance, body condition score and reproductive performance?
- Question 2: What are the specific effects of different fat supplements on the reproductive system?
- Question 3: Can fat supplementation have an effect on progesterone clearance rate during a feed deficit?

## 3. The experimental studies:

**Question 1: Study 1**

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 a supplement containing 12g of CLA or 60 g per day of calcium salts of palm fatty acids (CSFA; control) from parturition until 60 days in milk. The 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.



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**Question 1: Study 2**
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 (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.

**Question 2: Study 3**
Forty-eight early lactation Holstein-Friesian cows (21 primiparous, 27 multiparous) were used in a completely randomized block design. Cows were fed the same basal TMR diet and received one of four fat supplements: (i) palmitic acid (16:0 fatty acid; Control), (ii) flaxseed (rich in 18:3 n-3 fatty acid; Flax), (iii) conjugated linoleic acid (a mixture of cis-9, trans-11 and trans-10, cis-12 isomers; CLA), and (iv) fish oil (rich in 20:5 and 22:6 n-3 fatty acids; FO). All lipid supplements were formulated to be isolipidic; palmitic acid was added as necessary to provide a total lipid supplement intake of 500 g/day. Cows were synchronized to be in estrus on Day 15 of dietary treatment. All antral follicles were counted, and dominant follicles, subordinate follicles and corpora lutea were measured daily via transrectal ovarian ultrasonography for one complete estrous cycle. Blood samples were collected daily, and selected samples were analyzed for progesterone, estradiol, insulin-like growth factor-1, insulin, cholesterol and non-esterified fatty acids. Estrus was synchronized a second time, and liver and endometrial biopsies were collected on Day 7 of the estrous cycle. Gene expression was evaluated for a number of genes involved in prostaglandin synthesis (endometrium) and fatty acid uptake and utilization (liver).

**Question 3: Study 4**
Forty mid- to late-lactation Holstein-Friesian dairy cows were used in a completely randomized block design, with a 2 x 2 factorial arrangement of treatments. Cows were assigned to receive 1 of 2 pasture allowances (ad libitum allowance [AL], 9.5 kg dry matter per day, or restricted allowance [R] 7 kg dry matter per day) and 1 of 2 fat supplementation treatments (750 g per day saturated fat [F] or no fat supplement [NF]). All cows received an additional 4 kg per day of concentrate. Grass dry matter intake (GDMI) was measured 5 wk after the initiation of dietary treatment. Cows were treated with prostaglandin F2 (PGF2 ) to eliminate the endogenous source of P4, and two intravaginal progesterone-releasing devices (CIDR) were inserted into each cow for a period of 8 days. Regular blood samples were taken before and after the removal of the intravaginal progesterone-releasing devices, and analyzed for P4 concentrations.

## 4. Main results:

**Study 1**
The 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 CLA, but circulating concentrations of insulin, glucose, IGF-I, BHBA and progesterone were not affected. There was no effect of 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 CLA supplemented cows provided a strong rationale to conduct Study 2 with greater cow numbers to test effects on reproductive performance. Milk samples were collected three times per week to determine progesterone concentrations, which allowed determination of the postpartum interval to resumption of cyclicity

**Study 2**
The LE-CLA treatment resulted in a decrease in milk fat concentration (36.9 ± 0.06 g/kg vs. 30.7 ± 0.06 g/kg for control and LE-CLA, respectively) and yield (0.91 ± 0.02 kg/d vs. 0.84 ± 0.02 kg/d for control and LE-CLA, respectively); however, milk yield was increased by LE-CLA supplementation (24.7 ± 0.7 kg/d vs. 27.2 ± 0.7 kg/d for control and LECLA, respectively), resulting in no overall difference in milk energy output. No effect of LE-CLA was observed on any estrous cycle characteristics or measures of reproductive performance.

**Study 3**
Fat supplementation had little effect on follicle development. Cows receiving supplementary n-3 fatty acids had lesser plasma progesterone (P4) and smaller corpora lutea than cows receiving the CLA or Control supplements. Effects of fat supplementation on the endometrial expression of genes involved in PG synthesis were minor. Hepatic expression of SREBF1, ASCL1 and FABP1 was reduced by FO supplementation. Reduced plasma P4 in n-3 supplemented cows may lead to a suboptimal uterine environment for embryo development and hence reduced fertility compared to cows receiving the control or


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CLA supplements.

**Study 4**
The half-life (t½) and metabolic clearance rate (MCR) of P4 was calculated for each cow. There was no effect of GDMI or fat supplementation on the t½ or MCR of P4. There was a tendency for an interaction between GDMI and fat supplementation on the t½ of P4; cows on the restricted-F diet tended to have a longer P4 t½ than cows on the ad libitum-F diet. It was concluded that greater alterations in GDMI than achieved in the current study are required to change P4 metabolism. A combination of fat supplementation and restricted feeding slows P4 clearance, which may have beneficial implications for fertility.

## 5. Opportunity/Benefit:
The main benefit of the research undertaken is to highlight the nutritional value of a primarily pasture-based diet. Little benefit will be gained by feeding fat supplements specifically to alter reproductive function on a primarily grass-based diet. Supplements should be fed as necessary in periods of grass deficit to manage body condition score.

## 6. Dissemination:
Results were presented at the following conferences and workshops:

- Agricultural Research Forum 2009 and 2011, Tullamore, Ireland.
- European Society for Domestic Animal Reproduction 2009, Ghent, Belgium.
- American Dairy Science Association Annual meeting 2010, Denver, CO, USA.
- British Society of Animal Science Annual Meeting 2010, Belfast, Northern Ireland.
- British Society of Animal Science Cattle Fertility Conference 2014, Westport, Ireland.

### Main publications:


- Hutchinson, I. A., R. J. Dewhurst, A. C. Evans, P. Lonergan, and S. T. Butler. 2012. Effect of grass dry matter intake and fat supplementation on progesterone metabolism in lactating dairy cows. Theriogenology 78:878-886.
- Hutchinson, I. A., A. A. Hennessy, R. J. Dewhurst, A. C. O. Evans, P. Lonergan, and S. T. Butler. 2012. 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. Journal of Dairy Science 95(5):2442-2451.
- Hutchinson, I. A., A. A. Hennessy, S. M. Waters, R. J. Dewhurst, A. C. Evans, P. Lonergan, and S. T. Butler. 2012. Effect of supplementation with different fat sources on the mechanisms involved in reproductive performance in lactating dairy cattle. Theriogenology 78(1):12-27.


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