DAIRY PRODUCTS RESEARCH CENTRE

MOOREPARK

END OF PROJECT REPORT 1999: DPRC No. 26

C L A

A Health-Promoting Component of Animal and Milk Fat

Dr. Catherine Stanton

Supplementation of cows' diet with full fat rapeseed, full fat soybean, and pulp'n'brew (by-product of brewers' grains rich in linoleic acid) all elevate CLA levels in milk. Rapeseed is the most effective, increasing CLA levels by over 50% when fed as a supplement to cows on pasture The CLA-enriched milk fat was cytotoxic towards mammary and colon cancer cells.

Teagasc
The Science of Farming and Food

Nutrition: Nutritional Attributes of Animal and Milk Fat (CLA)

(CLA - A Health-Promoting Component of Animal and Milk Fat)

ARMIS No. 4257

Project Team:
Catherine Stanton (Leader)
Fergal Lawless
John Murphy
Seamus Aherne
Rosaleen Devery*
Marianne O'Shea

*School of Biotechnology, DCU

The Dairy Products Research Centre
Moorepark, Fermoy, Co. Cork.

Teagasc acknowledges with gratitude financial assistance for this project from the Dairy Levy Fund and by the EU (Concerted Action FAIR-CT98-3671 and SM & T4 CT97-2144). Thanks also to the Dairy Production Division, especially Bernie O'Brien, Seamus Fitzgerald and Pat Dillon, and to Kieran Keogh.

ISBN: 1 84170 118 1
DPRC No. 26

Teagasc 19 Sandymount Avenue
Ballsbridge Dublin 4

EUROPEAN UNION
European Regional Development Fund

Summary and Conclusions

In the recent past, there has been considerable interest in the potential health-promoting properties of conjugated linoleic acid (CLA), a fatty acid produced naturally in ruminant animals. CLA has been shown to be a very effective anti-cancer agent in animal models and cell culture studies, as well as being capable of retarding the initiation and progression of heart disease (atherosclerosis). It has also been shown to have potential as a growth promoter and is capable of improving feed efficiency. Hence from a human health viewpoint, it appears desirable to increase CLA levels in foods to protect against disease and enhance general health and well-being. The primary sources of CLA are animal fats (including dairy fats) derived from ruminant animals while vegetable fats and oils contain significantly lower levels.

This project was aimed at enriching the CLA content of dairy foods through animal dietary manipulation, and milk fat fractionation.

Main Conclusions and Achievements

- Supplementation of cows' diet with full fat rapeseed, full fat soybean, and pulp'n'brew (by-product of brewers' grains rich in linoleic acid) all significantly elevated CLA levels in milk. Rapeseed proved the most effective, increasing CLA levels by over 50% when fed as a supplement to cows on pasture. Milk output and composition were not affected.

- Although CLA levels were higher in late lactation in both Spring and Autumn calving herds, the dominant influence on CLA levels was the availability and quality of grazed pasture.

- CLA levels vary significantly in manufacturing milk over the season, ranging from a low of 5.5mg/g fat in March to three times this level (16 mg/g fat) in May, reflecting grass quality and availability.

- A milk fat fraction (soft) enriched in CLA (by over 60% compared with the parent fat) was produced using laboratory scale 'dry fractionation' of butter oil.

- In in vitro studies, CLA-enriched milk fat proved cytotoxic (lethal) towards human mammary cancer cells. It is thought that while acting as an anti-oxidant in the liver, CLA acts as a pro-oxidant in a variety of cancer cell lines.

- A survey of the CLA and trans-fatty acid content of a large range of foods has revealed that Irish foods have a relatively high CLA content, likely due to the relative importance of pasture grazing in this country.

Research and Results

Dietary supplementation

Dietary supplementation of cows with full fat rapeseeds and soybeans, which are rich in

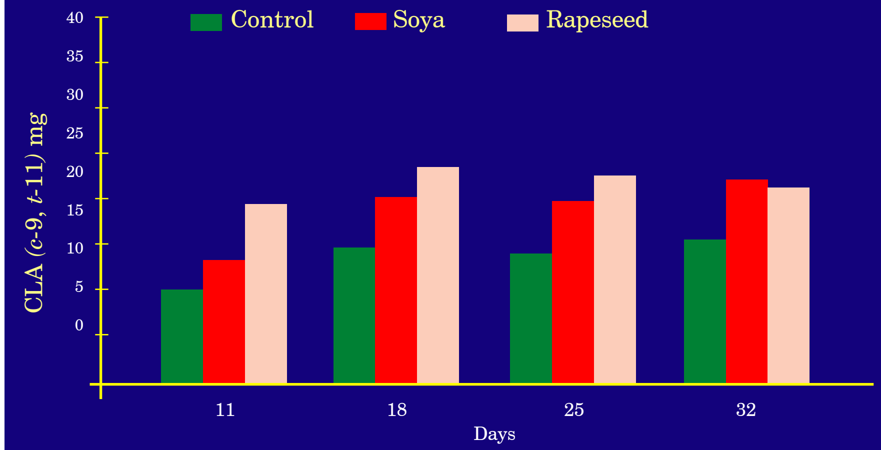
C18:1 and C18:2 fatty acids respectively, was investigated as a means of elevating milk fat CLA levels. Cows supplemented with a high (1.65 kg/cow/day) and medium (0.825 kg/cow/day) full fat rapeseed diets for eight weeks were compared with a control group of cows on pasture without supplementation.

The level of CLA in the milk from the high supplement diet was significantly higher than that on medium supplement, which in turn was significantly higher than the control group.

In a follow-up study the effects of supplementing cows on pasture with both full fat rapeseed and full fat soybean were compared using an unsupplemented group of cows on pasture as control *(Fig 1)*.

The CLA content of the milk fat of both supplemented herds was significantly higher than the unsupplemented control herd. Furthermore, the rapeseed-supplemented herd produced milk with a significantly higher CLA content than the soybean-supplemented herd, after 18 days of the feeding trial. The CLA levels were increased by 53% and 34% in the milk fat of the full fat-rapeseed and full fat soybean supplemented herds, respectively, at 18 days of the feeding trial.

Fig 1 **Milk fat CLA content increases with rapeseed-soybean supplementation**



48 dairy cows were blocked into groups of 3 according to: lactation number, stage of lactation and milk yield. Dietary Treatments were as follows:

* Control: *ad lib* pasture
* *ad lib* pasture + crushed full fat soybean (650g/cow/day soybean oil), and
* *ad lib* pasture + crushed full fat rapeseed (660g/cow/day rapeseed oil)

Milk yield and milk constituent yields were not affected by supplementation with either full fat rapeseeds or soybeans.

The influence on milk fat CLA content of supplementing lactating dairy cows with *pulp'n'brew* (a by-product mixture of brewers' grains) and sugar beet pulp, in dry matter proportions of 0.65 to 0.35 was investigated. For this study, forty-two Autumn calving cows were blocked into pairs according to days in milk (DIM) and pre-experimental milk yields and assigned at random to dietary regimes over three periods, using *pulp'n'brew* supplementation of either silage, Autumn grass or Spring grass.

The milk fat CLA concentrations were significantly higher when cows were fed silage supplemented with pulp'n'brew, compared with silage alone. Intake of Spring grass resulted in a 2.1-fold increase in milk fat CLA concentrations over cows receiving Autumn grass. This study demonstrated that ***pulp'n'brew,*** *which was higher in linoleic acid content than Autumn grass, concentrates and silage was more effective than these in elevating milk fat CLA concentrations but less efficient than Spring grass.*

Seasonal factors and calving patterns

In Ireland, approximately 85% of manufacturing milk is produced from Spring calving herds on pasture. Hence the levels of CLA in manufacturing milk were monitored over a full manufacturing season. The CLA content of milk varied throughout the Summer season, from a low of 5.5 mg/g fat in March to a high of 16 mg/g fat in May. Highest CLA levels were observed during May and September, which coincided with periods of lush grass supply. However, in a parallel study grass allowance was shown to have a significant effect on CLA levels *(Fig. 2)*.

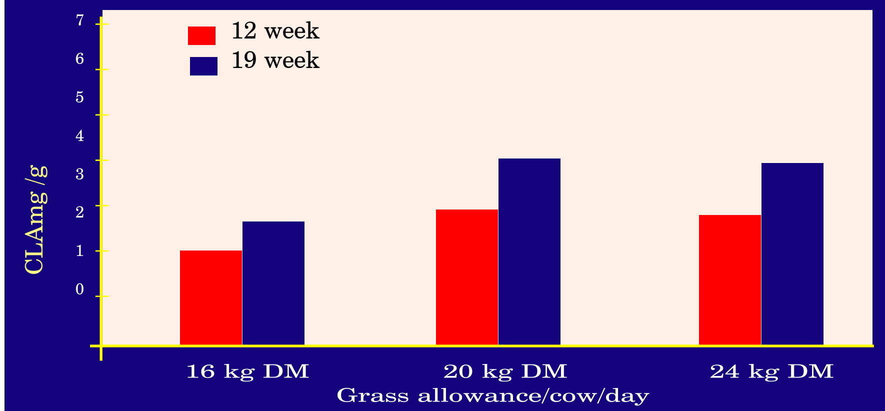
CLA was significantly lower when the grass allowance was at the lowest level (16 kg grass DM/cow/day) compared with the higher levels (20 kg and 24 kg/cow/day).

The influence of Spring compared with Autumn calving patterns and stage of lactation on bovine milk fatty acid profiles and CLA content were also examined. Spring calving cows receiving pasture throughout 80% of their lactation produced higher milk fat CLA concentrations (from 0.5 - 2.7 g/100 g FAME) than Autumn calving cows (0.3 - 1.7 g/100 g FAME) receiving *ad libitum* silage during approximately 50% of lactation and *ad libitum* pasture during the remainder of lactation.

The CLA content was higher in late lactation milk compared with early lactation milk in both herds. There were no significant differences in milk yield or milk constituent yields between the herds.

Milk fat CLA content was also studied in relation to Spring and Autumn calving patterns receiving contrasting dietary treatments. To achieve this, two herds, each of 26 cows, referred to as the Spring and Autumn calving herds were established, both having mean lactation numbers of three. The Spring herd calved in January to March and the Autumn herd calved in September to November. The Spring herd was sampled at seven time points (10, 19, 93, 137, 155, 227 and 270 DIM) during this lactation. The Autumn herd sampling covered portions of two lactations, and corresponded to 151, 276 and 26, 33, 54, 75 and 90 DIM, respectively. Composite milk samples of the a.m. and p.m. milkings were collected

Effect of Grass Allowance on milk fat CLA



* From indoor feeding to pasture increases CLA 2-3 fold
* Increase in grass intake increases CLA
* Early grass growth increases CLA
* Grass variety influences CLA

Fig 2

separately to form a single bulk sample from each of the Spring and Autumn herds on a regular basis throughout the study and the fat was extracted in duplicate for analysis of fatty acid profiles and CLA concentrations. The grass silage fed had a dry matter digestibility of 755 g/kg in 1996 and 806 g/kg in 1997. The concentrate offered contained (kg/1000 kg) maize gluten 250, citrus pulp 250, barley 200, rapeseed meal 140, fish meal 75, cane molasses 70 and minerals/vitamins 15 and had the following chemical composition (g/kg dry matter) crude protein 173, crude fibre 82, oil 37 and ash 88. Throughout lactation, Spring calving cows produced higher milk fat CLA concentrations (from 0.5 - 2.7 g/100 g fatty acid methyl esters (FAME)) than Autumn calving cows (0.3 - 1.7 g/100 g FAME); the former having spent 80% and the latter 50% of lactation on pasture. The CLA content was higher in late lactation milk compared with early lactation milk in both herds. There were no significant differences in milk yields or milk constituent yields between the herds.

This study revealed that milk fat CLA concentrations from these two herds were substantially different at similar stages of lactation and were higher from cows at pasture compared with those on silage and concentrates. The data indicate that the elevation in the concentration of CLA in milk was due primarily to the pasture feeding regime and that herds on pasture produced milk fat higher in CLA than herds at the equivalent stage of lactation which were fed silage.

Breed effect

The influence of animal breed on the levels of CLA in milk was investigated using three breeds of cows (Irish Holstein Friesian-medium genetic merit, Dutch Holstein Friesian-high genetic merit, Montbeliardes and Normandes) on pasture.

Milk fat CLA levels from Montbeliardes were significantly higher than high genetic merit Holstein Friesians, while the CLA content of milk fat from Normandes, medium and high genetic merit Holstein Friesians was not significantly different.

Dry fractionation

Another approach to CLA enrichment in milk fat, involving dry fractionation was examined as a potential alternative means of developing CLA-enriched milk fat fractions. Milk fat consists of a mixture of triglycerides that exhibit a broad and variable melting range, from approximately -40°C to +40°C. This allows the crystallising out of a series of triglycerides at temperatures below their melting points by controlled cooling of the melt. Since the process does not use solvents, it is known as 'dry fractionation' and has resulted in the production of food grade ingredients for products such as spreadable butters, chocolate and bakery products. The effect of laboratory scale dry fractionation on CLA enrichment of milk fat fractions was investigated using anhydrous milk fat (AMF), which was fractionated into hard and soft fractions using controlled cooling and agitation. Fractionation of milk fat pre-melted at 60°C using a temperature programme of 33°C to 10°C and a cooling rate of 0.58°C h^{-1} yielded a soft fraction containing 63.2% more CLA (2.22 g 100 g^{-1} FAME), which was also enriched in polyunsaturated fatty acids, compared with the parent fat. Agitation following fractionation was found to have a negative effect on the CLA content of the soft fraction, while refractionation of the soft fraction did not increase the yield of CLA. Following dry fractionation, the vaccenic acid content of the CLA-enriched milk fat fraction was increased by 28%, compared with the parent fat. The concentrations of all saturated fatty acids analysed were elevated in the hard fraction and similar in the soft fraction to the parent AMF. Conversely, the concentrations of all unsaturated fatty acids analysed were higher in the soft fraction and similar or slightly reduced in the hard fraction, compared with the parent AMF.

This study demonstrates that fractionation of milk fat resulted in a milk fat fraction enriched in CLA content, and provides an alternative method to approaches involving dietary oil supplements, which also lead to elevated milk fat CLA and vaccenic acid contents.

Processing and storage effects

The influence of processing and storage on the concentration of CLA in whole milk powder was investigated. The milk was standardized (to yield whole milk powder containing 26% fat) and pre-heated at low (75°C x 10 sec) and high (120°C x 2 min) heat. Milk standardization was achieved by removal of a milk fat fraction by centrifugation to yield a percent fat content (w/v) which was equivalent to 26% fat in dry matter. Milk concentrates underwent 2-stage homogenization (80 bar, first stage; 20 bar, second stage) prior to spray-drying in a pilot-scale Anhydro Lab 3 spray-drier, with pneumatic nozzle atomization. The powders were subsequently sachet-packed in foil-lined sample bags or vacuum-packed in foil bags using a Webomatic vacuum packer and stored under ambient (15°C) or 'hot room' (30°C) conditions in the dark up to 12 months. *Powders manufactured from milks produced by herds on pasture supplemented with full fat rapeseeds and soybeans were significantly higher (50% approx.) than those from the control herd - supplemented with sugar-beet pulp.* The powders from the rapeseed and soybean supplemented herds had CLA contents of 16.1 - 17.9 mg/g fat and 14.6 - 16.7 mg/g fat respectively, compared to the control herd values of 10.9 - 12.2 mg/g fat. However, the process of powder manufacture reduced the levels of CLA in the milk fat by up to 23% compared with the unprocessed milk.

CLA levels in specific powders which were sachet and vacuum packed decreased by relatively little (up to 2.1 mg/g fat) following 44 wk of storage at both 15°C and 30°C. In sachet-packed low heat powders manufactured from milk produced from a soybean supplemented herd, the CLA levels decreased by 10% and 30% respectively, following 44 wk of storage at 15°C and 30°C. On the other hand, CLA decreased by only 3.1 - 6.5% in both high heat vacuum and sachet-packed and low heat vacuum packed powders stored at either 15°C or 30°C.

The sachet-packed *low heat* powders manufactured from milk produced by the soybean supplemented herd and stored at 15°C and 30°C exhibited higher PV values (indicator of lipid oxidation) (3.3 and 8.2 mEq 0_2/kg fat respectively) than *high heat*, vacuum and sachet-packed and *low heat* vacuum packed powders stored at 15°C and 30°C (1.6 - 3.3 mEq 0_2/kg fat).

Effects of CLA on human mammary cancer cells

It is now accepted that CLA is produced endogenously from vaccenic acid (*trans*-11 $C_{18:1}$), a biohydrogenation intermediate in bovine mammary tissue by the action of delta-9 desaturase, as well as the direct production of CLA during the biohydrogenation of dietary linoleic to stearic acid in the rumen. Although the contribution of the CLA precursor trans-

vaccenic acid to CLA status in humans is not known, the delta-9-desaturase genes have been detected in human tissues. In a study by Ip and co-workers in 1999, CLA- (and vaccenic acid) enriched butter fed to rats (obtained by feeding cows plant oils rich in C_{18} polyunsaturated fatty acid CLA precursors) offered the same anticarcinogenic activity as did the synthetic CLA.

Milk enriched in CLA (and vaccenic acid) through the use of supplements, and dry fractionation (above) was evaluated *in vitro* for cytotoxicity toward human mammary cancer cell (MCF-7). Cell number decreased by $\sim 90\%$ ($p < 0.05$) and lipid peroxidation increased 15-fold ($p < 0.05$) following incubation of MCF-7 cells with increasing levels of milk fat CLA, between 0 and 22.6 ppm for 8 days.

A dose-dependent decrease in cell proliferation was seen in human cancer cells treated with bovine milk fat enriched with CLA. All milk fat samples significantly lowered ($p < 0.05$) cell number to approximately 7 - 9% of control cells at a CLA concentration of 20 ppm CLA in the incubation medium, following 8 days of incubation *(Fig. 3)*. The 0 control cells were incubated in the absence of milk fat (red bar).

However there was no significant difference between the final cell numbers (8.3 - 11.7 x 10^4 cells) for all milk fat treatments *(Fig.3)* - control milk fat (from cows on pasture), milk fats from soybean and rapeseed supplemented cows, and CLA-enriched soft fraction obtained by dry fractionation.

Growth suppression of MCF-7 cells by milk fat CLA was accompanied by uptake into cell membrane phospholipids, pro-oxidant effects and enhancement of antioxidant defense enzymes, superoxide dismutase, catalase and glutathione peroxidase. CLA accumulated in cell membranes more efficiently from milk fat than the pure *c*9, *t*11 synthetic CLA isomer.

These results suggest that CLA in milk fat is cytotoxic towards mammary cancer cells and the results demonstrate the potential of CLA-enriched milk-fat-containing foods in cancer prevention in humans.

Incubation of MCF-7 cells with milk fat (to yield 20 ppm CLA) for 8 days significantly reduced cell numbers to approximately 7 - 9% of control cells, incubated in the absence of milk fat for 8 days.

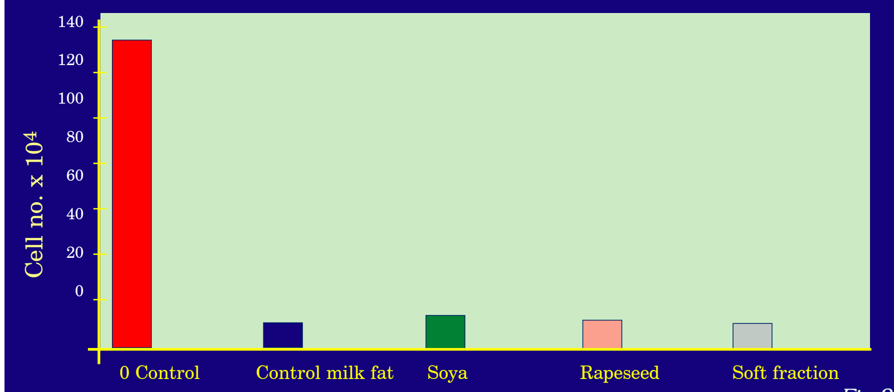


Fig 3

Publications

Stanton, C., Lawless, F., Kjellmer, G., Harrington, D., Devery, R., Connolly, J.F. and Murphy, J. (1997). Dietary Influences on Bovine Milk *cis-9, trans-11*-Conjugated Linoleic Acid Content. *Journal of Food Science*, 62:1083-1086.

Stanton, C., Lawless, F., Murphy, J. and Connolly, J.F. (1997). Conjugated Linoleic Acid - A Marketing Advantage for Animal Fats. In *'Animal Fats: BSE and After'* (Ed. Berger, K.). Publishers P.J. Barnes and Associates, Bridgewater, UK, pp. 27-41.

Lawless, F., Murphy, J., Harrington, D., Devery, R. and Stanton, C. (1998). Elevation of Conjugated *cis-9, trans-11*-Octadecadienoic Acid in Bovine Milk Because of Dietary Supplementation. *Journal of Dairy Science*, 81, 3259-3267.

O'Shea, M., Devery, R., Lawless, F. and Stanton, C. (1998). Conjugated Linoleic Acid in Bovine Milk Fat: A Food-Based Approach to Chemoprevention. *Trends in Food Science*, 9: 192-196.

Lawless, F., L'Escop, P., Stanton, C. and Murphy, J. (1999). Effect of Breed on Bovine Milk Fat CLA Content. *Livestock Production Science,* 62: (1), 43-50.

O'Shea, M., Stanton, C. and Devery, R. (1999). Antioxidant Enzyme Defence Responses of Human MCF-7 and SW480 Cancer Cells to Conjugated Linoleic Acid. *Anticancer Research* 19: 1953-1960.

Ip, C., Banni, S., Anigoni, E., Carta, G., McGinley, J., Thompson, H.J., Barbano, D. and Bauman, D. (1999). Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J. Nutr.*, 129: 2135 - 2142.

Lawless, F., Murphy, J.J., Fitzgerald, S., O'Brien, B., Devery, R. and Stanton, C. (2000). Dietary effect on bovine milk fat conjugated linoleic acid content. *British Society of Animal Science*, occasional Publication No. 25 (In Press).

O'Shea, M., Devery, R., Lawless, F., Keogh, K. and Stanton, C. (2000). Enrichment of the conjugated linoleic acid content of bovine milk fat by dry fractionation. *Int. Dairy Journal* 10, 289-294.

O'Shea, M., Devery, R., Lawless, F., Murphy, J. and Stanton, C. (2000). Milk fat conjugated linoleic acid (CLA) inhibits growth of human mammary MCF-7 cancer cells. *Anticancer Letters* (In Press).

For further information, please contact Dr. Catherine Stanton


Dr. Catherine Stanton

DAIRY PRODUCTS RESEARCH CENTRE
Moorepark, Fermoy, Co. Cork, Ireland
Tel: +353 (0) 25 42222 - Fax: +353 (0) 42340
E-mail: reception@moorepark.teagasc.ie