# Adding value to milk by increasing its protein and CLA contents

## End of Project Report

## Project 5400

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# 1. Summary

Five experiments were undertaken in this project; one on mid-summer milk protein and four on milk CLA content.

The mid-summer milk protein study was undertaken on 34 commercial dairy farms in 2005 to evaluate the influence of dietary and management variables on milk protein content in mid-season. Data on grass composition, genetic merit of the herds and milk protein content were collected and analysed by multiple regression. Both calving date and genetic merit for milk protein content were significantly associated with milk protein content and were used as adjustment factors when evaluating the association between measures of grass quality and milk protein content. Milk protein content was associated with grass OMD ($P = 0.04$) and NDF content ($P = 0.02$) but not with CP content ($P = 0.80$). It is concluded that herds calving earlier, with a greater genetic merit for milk protein content and consuming better quality pasture would have greater milk protein contents in mid-season.

The objective of the first CLA study was to assess the benefit of a pasture diet supplemented with sunflower oil, compared with an indoor diet for conjugated linoleic acid (*cis*-9, *trans*-11 CLA – hereafter referred to as CLA) enrichment of milk suitable for product manufacture. A group of 40 autumn-calving dairy cows were assigned to either a control group (indoor feeding on grass silage ad-libitum and 6 kg/day of a typical indoor concentrate) or an experimental group (on pasture, being fed 6 kg of a supplement containing 100 g/kg of sunflower oil per day). These diets were fed for 16 days, during which time milk was collected for pilot-scale hard cheese manufacture. The pasture-based diet with sunflower oil resulted in a significant effect on the milk fatty acid CLA content. The concentration of *cis*-9, *trans*-11 CLA in the milk produced from cows on this diet increased to 2.22 g/100 g of fatty acid methyl esters (FAME) after 14 days, compared with 0.46 g/100 g of FAME in milk produced on the control indoor diet. The outdoor diet also resulted in milk with significantly higher concentrations of C18:1 *cis9* (oleic acid) and C18:2 *cis9, cis12* (linoleic acid) and the yield of milk and protein was also significantly greater on the outdoor diet. Thus, a pasture-based diet supplemented with an oil source rich in linoleic acid resulted in an enhanced CLA content of bovine milk fat and greater yields of milk and protein, compared with an indoor grass silage-based diet.

The principal aim of the second CLA study was to determine if relatively low levels of fish oil or sunflower oil, either alone or in combination, offered to dairy cows on pasture would increase the CLA concentration in milk, above the relatively high levels achieved on pasture only. Forty autumn-calved cows on a diet of grazed grass were assigned to 4 supplementation treatments: (i) No supplement (P), (ii) 255 g/day of sunflower oil (SO), (iii) 255 g/day of sunflower oil + 52.5 g /day of fish oil (SOFO), and (iv) 105 g /day of fish oil (FO). The fish oil was supplied in a proprietary product called Omega-3 Supplement which is a mixture of marine oils and an extracted oilseed meal and contains 500 g/kg of oil. The oils were fed in a concentrate mixture, which was offered at a rate of 3.0 kg/cow per day. The production of the cows was measured for 54 days and the milk fatty acid composition was determined on day 0 (immediately before the supplements were introduced) and on days 14, 28 and 42 after the treatments were imposed. Supplementation increased the yield of milk ($P < 0.01$), protein ($P < 0.05$) and lactose ($P < 0.001$), decreased milk fat ($P < 0.05$) and protein ($P < 0.01$) concentrations and increased ($P < 0.01$) lactose concentration. Type of oil did not significantly affect any production variable. The concentration of C18:1 *trans* 9 + C18:1 *trans* 11 (mainly C18:1 *trans* 11 – hereafter referred to as vaccenic acid) ($p < 0.001$) and CLA ($P < 0.01$) were greater on

supplemented treatments than on P and the concentration of both were greater ($P < 0.05$) on FO than on SO. The results confirm that the concentration of CLA can be increased further, from an already relatively high concentration in milk from pasture, by offering supplements containing a low level of fish oil either alone or in combination with sunflower oil.

The objectives of the third study were to investigate the underlying mechanisms for the reported elevation in milk CLA production associated with grazed grass (G) compared to zero-grazed grass (ZG) and grass silage (GS), and to identify the important variables contributing to the milk CLA response. Six rumen-cannulated Holstein-Friesian cows in early lactation were assigned to three treatments – G, ZG and GS harvested from the same perennial rye grass sward in a 3×3 Latin square design with three 21-day periods. Grazing animals were offered 20 kg dry matter (DM)/cow per day. Indoor animals were offered *ad libitum* grass or silage. A concentrate at a rate of 3 kg/day was also offered to all cows. Rumen, plasma and milk samples were collected in the third week of each period. Dry matter intakes were significantly lower in GS with no difference between G and ZG. Milk yield was significantly different across the treatments with higher values recorded in G and lower values in GS. Milk fat and protein contents were significantly lower in GS with no difference between G and ZG. Intake of substrate (18:2n-6 + 18:3n-3 in g/day) was significantly different across the treatments (G: 433; ZG: 327; and GS: 164). Vaccenic acid concentrations were significantly different across the treatments in rumen (G – 12.30%, ZG – 9.31% and GS – 4.21%); plasma (G – 2.18%, ZG – 1.47% and GS – 0.66%) and milk (G – 4.73%, ZG – 3.49% and GS – 0.99%). Milk CLA concentrations were significantly higher in G (2.07%) compared to ZG (1.38%) and GS (0.54%) but CLA concentrations in rumen and plasma were extremely low (≤ 0.17%). Milk desaturase index based on the ratio *c*9-14:1/14:0 was not different across the treatments. Milk CLA yield per 100 g of substrate intake (efficiency) was 2.23, 1.50 and 0.62 g in G, ZG and GS, respectively, suggesting that G cows were more efficient than ZG and GS cows in milk CLA production. Step-wise regression analysis of a group of variables at $P \leq 0.09$ revealed that plasma vaccenic acid accounted for 95% of the variation in milk CLA production. Milk desaturase index did not enter into the model. Overall findings suggest that substrate intake strongly influenced milk RA production but it was not the only factor involved. There were differences in efficiency of milk CLA production which appears to depend on the factors regulating ruminal VA production and its supply to the mammary tissue

The objective of the fourth CLA study was to evaluate the response of high and low CLA cows to a supplement that is known to enhance CLA concentrations to determine if it would be possible to produce milk with a very high CLA concentration from selected cows offered a CLA enhancing supplement. Forty autumn calving cows selected from a total herd of 70 were used in the study. Milk samples were taken from all of the 70 cows 4 and 8 weeks after calving while on indoor diets of grass silage and concentrates for CLA analysis. A further milk sample for CLA analysis was taken from the cows in early April when they were all on a diet of grazed grass. The cows were ranked from highest to lowest on the basis of their CLA concentrations. The highest 20 and lowest 20 were allocated to a high CLA and low CLA group, respectively. Within these groups cows were then blocked into the following two treatments on the basis of CLA concentration and milk yield: (i) Pasture ad-libitum (Control) and (ii) Pasture ad-libitum plus 3.0 kg/day of supplement containing 105g/day of fish oil (Omega 3 supplement – UFAC, UK Ltd.) Cows grouped on the basis of their CLA content did not differ in their production characteristics. High milk

CLA cows had a greater *c9*-C14:1/C14:0 ratio indicating that greater mammary desaturase activity may be a contributory factor in their higher CLA. Groups of cows selected for their high and low CLA concentrations when on the same diet, did not respond differently when supplemented with fish oil which is known to increase milk CLA content. Therefore selecting high CLA cows and supplementing with a CLA-promoting diet will not result in the production of milk with extremely high CLA concentrations.

# 2. Introduction

Despite increasing slowly in recent years (from a mean of 32.1 g/kg in 1997) Irish milk protein content is still lower (mean of 33.4 g/kg in 2007) than in most other European countries and is substantially lower than in New Zealand. Also in Ireland there is a large seasonal variation (in 2007 from 31.0 g/kg in March to 36.5 g/kg in November) and this in combination with the overall low concentration is a disadvantage for the Irish dairy industry as a whole. It is perplexing for the industry that milk protein content remains at a relatively low and stable concentration in the months of June, July and August when it would be expected to be increasing. The reason for this has never been clearly explained but the poor digestibility of mid-summer grass has been proposed as a likely cause. Increasing milk protein content in this period would have a relatively large effect on the average annual protein content because approximately 36% of the annual milk supply is supplied in these months.

Dietary factors affecting milk protein content have been reviewed (Sporndly 1989; De Peters and Cant 1992; Murphy and O' Mara 1993 among others). Many discrete factors such as energy (concentrate) intake, lipid intake, starch and sugar in the diet, amino acid supply and forage type have been shown to modulate milk protein content but the potential effect of these factors in a high grass based system of milk production may be limited. However, the factors associated with the low mid-summer milk protein content need to be determined so that cost effective corrective strategies can be put in place.

Bovine milkfat contains a number of components, such as conjugated linoleic acid (*cis-9, trans 11* CLA – hereafter referred to as CLA), (C18:1 *trans 11* – hereafter referred to as vaccenic acid), sphingomyelin, butyric acid, ether lipids and vitamins that have demonstrated anticancer potential in animal models of carcinogenesis and in a variety of human cancer cell lines (Parodi 1999). CLA, is a natural component of milkfat and has several associated health promoting attributes, including anti-carcinogenic, growth promotion and anti-obesity activities. It is formed as a result of microbial biohydrogenation of dietary linoleic acid in the rumen by bacteria. Some of the CLA formed in the rumen is absorbed into blood and incorporated into milk fat and a portion is further biohydrogenated by rumen bacteria to vaccenic acid, which is also found in milk fat. Vaccenic acid is also a precursor of CLA, because CLA can be synthesised in the bovine mammary gland from vaccenic acid, in a reaction catalysed by delta 9-desaturase. It has been estimated that about 90% of CLA in milk from pasture arises via this pathway.

Foods rich in CLA and also in vaccenic acid contribute to tissue CLA, and therefore may contribute to the protective effects associated with dietary CLA. Milk and dairy foods, and meat from ruminant animals such as beef and lamb are rich sources of these beneficial fatty acids.

Because of the seasonal pattern of calving and milk production in Ireland grazed grass is the sole or principal component of the cow's diet for up to nine months of the year. It has been demonstrated by us, as well as others, that milk produced from pasture has a two to three fold higher concentration of CLA than milk produced indoors from silage and concentrates (Stanton et al 1997; Kelly et al 1998; Dhiman et al. 1999). Concentrations of CLA in Irish milk produced from pasture are approximately 15-16 mg/g of fat. We have shown how it can be further increased to 23-24 mg/g of fat by supplementing pasture with whole crushed rapeseeds (Lawless et al. 1998). In our studies it was shown that cow breed (Holstein-Friesian, Montbeliarde and Normande) had little effect on milk CLA content but there was approximately a seven-fold range

between individual cows within breeds on the same diet of pasture only (Lawless et al. 1999). Therefore, a number of issues in relation to increasing CLA have been addressed in this project: (i) the efficacy of different oil sources in increasing CLA at pasture (ii) the magnitude of increase in CLA that can be achieved in going from an indoor to a pasture based diet, (iii) the reasons for the difference in CLA content on grass and grass silage and (iv) the potential to produce milk at pasture with very elevated CLA concentrations from cows with "naturally" high CLA concentrations
Thus the two main objectives of this project were to determine the factors associated with milk protein concentration in mid-summer and to investigate potential further strategies to increase the CLA content of pasture produced milk.

# 3. Experiments

## 3.1 Experiment 1

### Introduction

The protein content of milk in Ireland is lower and more variable than in other European countries (Prospectus Report 2003). This is due to a combination of the high forage nature of the cows' diet and the seasonal calving profile of the vast majority of Irish dairy cows. The average protein concentration of manufacturing milk in Ireland in the calendar year 2007 was 33.4 g/kg, varying from 31.0 g/kg in March to 36.5 g/kg in November (data source – Central Statistics Office Ireland). Milk protein concentration increases gradually from March to May but in the months June, July and August declines somewhat or remains stable and is always lower than would be expected given the lactation stage of the majority of the cows at that time. The objective of this study was to determine what dietary and management factors influence milk protein concentration on commercial dairy farms in the mid-season period of June July and August.

### *Materials and methods*

A survey of grass quality, management, and genetic factors was undertaken on 36, geographically spread, commercial dairy farms in 2005. The location of the farms by county was: Cork 11, Limerick 6, Kerry 3, Clare 2, Cavan 2, Kilkenny 2, Laois 2, Monaghan 2, Tipperary 1, Meath 1, Westmeath 1 and Offaly 1. Farms were visited on three occasions at approximately 2-week intervals between mid-June and mid-August. Pre grazing sward height was measured using an electronic plate meter (Agrosystèmes, Choiselle, France) and grass samples representative of herbage being offered to the herd were harvested with a Gardena shears (Accu 60, Gardena International HmbH, Ulm, Germany). These samples were freeze dried and milled through a 1 mm sieve in a Cyclotec 1093 Sample Mill (Foss 265 Electric, DK-3400 Hillerod, Denmark) and analyzed for organic matter digestibility using the NDF cellulase technique (Morgan et al.1989), neutral detergent fibre by the method of van Soest et al. (1991) using Ankom equipment (Ankom Technology Corporation, NY, USA), crude protein by the method of Sweeney (1989) using a Leco FP 428 nitrogen analyser and ash content by burning in a muffle furnace at 550 ºC for 16 h. Herd milk protein concentrations for the weeks of grass sampling were obtained from the co-operative purchasing the milk from the farm, while data on genetic merit and calving pattern of the herds was provided by the Irish Cattle Breeding Federation. The descriptive statistics for grass composition, milk protein concentration and genetic merit of the herds for the farms used in the analysis are shown in Table 1

**Table 1** Descriptive statistics for grass composition, herd characteristics and genetic merit of the herds in the study

| Variable | Mean | Min | Max |
|---|---|---|---|
| Organic matter digestibility (g/kg DM) | 823 | 751 | 861 |
| Neutral detergent fibre (g/kg DM) | 422 | 351 | 514 |
| Crude protein (g/kg DM) | 197 | 116 | 265 |
| Ash (g/kg DM) | 84 | 65 | 111 |
| Pre-grazing sward height (cm) | 15.5 | 9.6 | 24.0 |
| | | | |
| Number of cows | 62 | 25 | 142 |
| Milk protein (g/kg) | 33.3 | 31.1 | 35.7 |
| Mean calving date | 24/2 | 11/2 | 29/3 |
| | | | |
| Economic breeding index | 31.8 | 15 | 52 |
| PD milk (kg) | 60.5 | -103 | 182 |
| PD fat (kg) | 3.14 | -0.8 | 6.8 |
| PD fat (%) | 0.02 | -0.04 | 0.08 |
| PD protein (kg) | 3.81 | -1.5 | 7.5 |
| PD protein (%) | 0.03 | -0.01 | 0.06 |

***Statistical Analysis***

The association between grass composition variables and herd milk protein content was analyzed using multivariate mixed models (PROC MIXED, SAS). A series of univariate analyses were initially carried out to determine the association between milk protein and a range of genetic and management factors (i.e. genetic merit for milk yield, milk fat and milk protein, mean calving date) and those found to be associated ($P<0.05$) with milk protein concentration were included as adjustment factors in each final model. The final models therefore consisted of the relevant grass variable, and the adjustment factors. Herd was included as a random effect to account for multiple visits per farm.

***Results***

Univariate analysis showed that herd milk protein concentration was significantly associated with herd genetic merit for milk protein concentration ($P = 0.01$) and calving date ($P = 0.03$) (Table 2). Therefore, these two variables were included as adjustment factors in the analysis to evaluate the association between milk protein concentration and measures of grass quality. Pre grazing height and grass CP content were not significantly associated with milk protein concentration. Grass OMD ($P = 0.04$) and NDF ($P = 0.02$) contents were found to be significantly associated with milk protein concentration (Table 3). The coefficient for the association between OMD and milk protein content was positive indicating that greater grass digestibility was associated with greater milk protein content and the coefficient for the association between NDF and milk protein content was negative indicating that less fibre content in the grass was associated with greater milk protein content. Based on the regression equation obtained from the data in this study [milk protein g/kg = 28.4 - (0.032 x calving day of year) + (20.502 x PD milk protein %) + (0.0729 x OMD)] a one-unit increase in grass OMD would result in an increase of 0.09 g/kg in milk protein

concentration assuming that calving day of year and PD for milk protein % remained constant.

**Table 2** The significance of relationships between genetic variables, management variables and grass variables and milk protein content measured on 3 occasions on farms in June, July and August.

| **Variable** | **Coefficient**[1] | **P-value** |
|---|---|---|
| Mean calving date | -0.35 | 0.03 |
| PD milk kg | 0.03 | 0.21 |
| PD protein kg | 1.48 | 0.07 |
| PD protein % | 251 | 0.01 |
| PD fat kg | 1.49 | 0.09 |
| PD fat % | 48 | 0.53 |
| | ***Coefficient*** [2] | |
| Pre-grazing height | -0.15 | 0.43 |
| Crude protein content | 0.04 | 0.80 |
| Organic matter digestibility | 0.73 | 0.04 |
| Neutral detergent fibre | -0.57 | 0.02 |

[1] Univariate analysis

[2] Adjusted for differences in mean calving date and genetic merit for milk protein concentration

***Conclusions***

In conclusion, higher milk protein concentration in Irish commercial herds in mid-season is associated with higher quality grass, earlier calving and higher genetic merit for milk protein concentration.

## 3.2 Experiment 2

***Introduction***

Many studies have been undertaken in an attempt to increase the vaccenic acid and CLA content in milk fat (for reviews see Stanton et al., 2003, and Khanal and Olson, 2004). To date, studies have indicated that the diet of the ruminant animal has a significant effect on CLA levels in milk fat. Lipid substrates that have been added to the bovine diet and proven to be successful for enhancing the CLA concentration in milk fat include plant oils rich in PUFA and fish oils rich in long-chain PUFA, the former through enhancement of biohydrogenation substrates, and the latter by affecting pathways of rumen biohydrogenation (Chilliard et al., 1999; Dhiman et al., 2000). Studies have also confirmed that pasture feeding significantly and rapidly increases milk fat CLA concentrations (Kelly et al., 1998) but the CLA-enriching effect of pasture feeding has not been conclusively characterized. The magnitude of the effect on milk CLA concentration of changing from an indoor to a pasture plus sunflower oil diet was evaluated in this study with a view to producing CLA-enriched milk for the manufacture of naturally enhanced CLA-rich dairy products.

***Materials and Methods***

Forty autumn-calving dairy cows were blocked on the basis of calving date and milk yield into pairs and were randomly assigned from within pairs to 2 treatment groups: 1) indoor feeding on grass silage ad libitum and 6 kg of a typical indoor concentrate (IC), and 2) pasture feeding plus 6 kg of supplement (PS) containing 100 g/kg of sunflower oil (Trilby Trading Ltd., Drogheda, Ireland). The cows had a lactation number of 3.8 (SD 1.35), were 241 d (SD 31.8) in lactation, and were yielding 23.3 kg of milk/cow per d (SD 3.26) when treatments commenced. Treatments were imposed for a total of 16 days. The IC consisted of (kg/t) 250 barley, 270 unmolassed sugar beet pulp, 170 maize gluten feed, 150 rapeseed meal, 100 soyabean meal, 30 lard, and 30 minerals plus vitamins. The sunflower oil supplement consisted of (kg/t) 670 unmolassed sugar beet pulp, 200 corn gluten feed, 100 sunflower oil, and 30 minerals plus vitamins. Vitamin E inclusion was 10-fold greater in the sunflower oil supplement at 150,000 IU/t. The indoor and sunflower oil concentrate supplements contained (g/kg of DM) 195 and 116 of CP, 271 and 410 of NDF, and 105 and 99 of ash, respectively. The grass silage feed contained (g/kg of DM) 162, 623, and 76 of CP, NDF, and ash, respectively, and had a DM digestibility value of 664. The pasture offered was composed principally of perennial ryegrass. The mean quantity of grass DM available at the start of each grazing during the experimental period was 2.22 t/ha. It contained (g/kg DM) 159, 400, and 83 of CP, NDF, and ash, respectively. All cows were on pasture full time for 88 days before treatments commenced and had received no concentrate supplement for the previous 48 days. Individual milk samples were taken from these cows at the evening milking on day 0, 7, and 14 after treatments commenced.

***Statistical analysis***

The performance data in the calendar week corresponding to d 6 to 12 on treatment, inclusive, were analyzed to compare treatments. Milk yield, constituent yields, and milk composition were analyzed using the GLM procedure (SAS Institute, Inc., Cary, NC). The least squares means reported here were adjusted for block, lactation number, calving date, and the appropriate pre-experimental variable. Fatty acids in individual cow milk samples on d 14 were analyzed by the GLM procedure of SAS, and the least squares means reported here were adjusted for block and d 0 values.

***Results***

*Production Data*

The mean values for the major milk constituents are detailed in Table 3. Yields of milk, protein, and lactose were greater ($P < 0.001$) for cows outdoors on the pasture-based sunflower oil treatment than those indoors on the grass silage-based treatment. Fat yield was unaffected by the treatments, but fat concentration was reduced ($P < 0.001$) in the PS compared with the IC (41.7 and 32.6 g of fat/kg of milk, respectively) treatment. Milk fat depression has previously been reported as a result of supplementation of the diet with oilseeds, fish oil, and high concentrate levels (for reviews see Davis and Brown, 1970; Bauman and Griinari, 2001). In the current experiment, the depression in milk fat concentration resulted from a dilution effect because of increased milk volume production. The milk protein concentration was higher ($P < 0.001$) on PS, and the lactose concentration was similar on both treatments. Such production differences have been observed previously, in which

grass silage and the grass from which it was produced have been compared directly (Keady et al., 1999).

**Table 3** Yields of milk, constituents and milk composition on the indoor grass/silage-based diet (IC) and the outdoor pasture-based diet with added sunflower oil (PS)

| | Treatment Group | | |
|---|---|---|---|
| Yield | Control (IC) | Pasture & Sunflower Oil (PS) | SEM |
| Milk (kg/d) | 17.3 | 23.5 | 0.47 |
| Fat (g/d) | 721 | 760 | 24.4 |
| Protein (g/d) | 552 | 824 | 17.3 |
| Lactose (g/d) | 800 | 1086 | 21.1 |
| Fat (g/kg) | 41.7 | 32.6 | 1.34 |
| Protein (g/kg) | 32.1 | 35.3 | 0.29 |
| Lactose (g/kg) | 46.2 | 46.4 | 0.28 |

*Fatty Acid Composition of the Milk Fat*

The fatty acid composition of the raw milk from cows on the IC and PS diets at d 14 of the trial are detailed in Table 4. The PS diet resulted in an increase in linoleic acid in the milk fat ($P < 0.001$). This was due to high levels of linoleic acid in the sunflower oil. Other fatty acids were also affected in the milk from the PS diet, compared with that produced on the IC diet, as a result of the combination of pasture feeding and sunflower oil supplementation. There was a decrease ($P < 0.01$) in the concentration of short-chain fatty acids, with the exception of butyric acid (C4:0), which remained constant, and there was an increase ($P < 0.01$) in the concentration of long-chain fatty acids, with the exception of linolenic acid, which remained constant between the IC and PS diets. There was a decrease ($P <0.001$) in the palmitic acid (C16:0) concentration and an increase in the stearic acid (C18:0) concentration in the PS milk compared with the IC milk ($P < 0.001$). There were large increases ($P < 0.001$) in the concentration of vaccenic acid, and in the concentrations of oleic acid (C18:1 *cis 9*) in the PS milk compared with the IC milk. Although this experimental design could not separate the impacts of pasture and the supplement separately the results clearly demonstrate that the PS diet (pasture plus supplement) was effective at increasing the concentration of the biologically active CLA isomer in the raw milk. At day 14, the concentration of CLA in the PS milk was almost 5-fold greater than that in the IC milk (2.22 and 0.46 g/100 g of fatty acid methyl esters (FAME), respectively, $P< 0.001$).

**Table 4** Effect of treatment on the milk fatty acid composition of cows indoors on control diet (IC) and cows outdoors on pasture receiving sunflower oil supplementation (PS), at day 14

| Fatty acid (g/100 g FAME) | Treatment Group | | SEM | *P* value[1] |
|---|---|---|---|---|
| | Control (IC) | Pasture & Sunflower Oil (PS) | | |
| C4:0 | 1.46 | 1.51 | 0.04 | 0.396 |
| C6:0 | 1.50 | 1.22 | 0.04 | <0.01 |
| C8:0 | 1.18 | 0.85 | 0.04 | <0.001 |
| C10:0 | 2.92 | 1.86 | 0.08 | <0.001 |
| C12:0 | 3.56 | 2.30 | 0.08 | <0.001 |
| C14:0 | 12.82 | 9.35 | 0.17 | <0.001 |
| C14:1 total | 1.45 | 1.41 | 0.05 | 0.537 |
| C15:0 | 1.66 | 1.13 | 0.03 | <0.001 |
| C16:0 | 35.59 | 21.62 | 0.43 | <0.001 |
| C16:1 total | 2.30 | 2.09 | 0.04 | <0.01 |
| C17:0 | 0.60 | 0.61 | 0.01 | 0.807 |
| C18:0 | 7.51 | 12.63 | 0.13 | <0.001 |
| C18:1 *t*9 + C18:1 *t*11[2] | 1.37 | 8.05 | 0.25 | <0.001 |
| C18:1 *t*13 + C18:1 *c*9[2] | 18.27 | 25.63 | 0.28 | <0.001 |
| C18:2 *c*9, *c*12 linoleic acid | 1.14 | 2.53 | 0.07 | <0.001 |
| C20:0 | 0.12 | 0.10 | 0.00 | <0.01 |
| C18:3 *c*9,*c*12,*c*15 linolenic acid | 0.53 | 0.53 | 0.01 | 0.740 |
| C18:2 *c*9, *t*11 CLA | 0.46 | 2.22 | 0.04 | <0.001 |

[1]The fatty acid levels have been statistically adjusted for block effects and d 0 levels of fatty acids.

[2]The peaks generated on the GC for C18:1 *t*9 and C18:1 *t*11 vaccenic acid are very close and thus are difficult to separate accurately, so to avoid subjectivity on the division of the peaks, these two peaks are counted as one, with the C18:1 *t*11 vaccenic acid being the major fatty acid present. This also occurs with C18:1 *t*13 and C18:1 *c*9 oleic acid, with C18:1 *c*9 oleic acid being the major peak.

### *Conclusions*

This study illustrates that the concentration of CLA is significantly greater in milk from cows on pasture supplemented with sunflower oil than in milk from cows indoors on a grass and silage-based diet. The concentration of CLA increased in the sunflower oil-fed group on pasture by d 14 of the trial, to 2.22 g/100 g of FAME, compared with 0.46 g/100 g of FAME in the milk of the indoor silage and concentrate fed group. The pasture based diet also results in greater yields of milk, protein and lactose, greater milk protein content and a similar fat yield to the indoor diet.

## 3.3 Experiment 3

***Introduction***

In studies published to date, fish oil either alone or in combination with vegetable oils has resulted in increased CLA contents in milk fat (Donovan *et al.*, 2000; AbuGhazaleh and Holmes, 2007). Fish oil supplementation can alter milk composition and in particular decrease milk fat concentration (Chilliard *et al.*, 2001). Therefore, it would be desirable to minimise the quantity fed while still obtaining the positive effect on milk CLA content. In previous studies it is estimated that up to 300g/cow per day of fish oil has been supplemented either alone or in combination with varying amounts of vegetable oils in order to effect an increase in CLA concentration. The aim of this study was to determine if low levels of fish oil either alone or in combination with sunflower oil, offered to dairy cows on pasture, would increase the CLA concentration in milk.

***Materials and methods***

*2.1 Animals and experimental treatments*

Forty autumn-calved cows were used in the study which was of 54 days duration between May and July. Cows were grouped into blocks of four on the basis of lactation number (lactation number 1, lactation number 2 and lactation number 3+), days in milk (202 ± 28.1) and pre-experimental milk yield (27.0 ± 3.4) and allocated at random from within blocks to one of the following supplementation treatments:

(i) No supplement (P)
(ii) 255 g/day of sunflower oil (SO)
(iii) 255 g/day of sunflower oil + 52.5 g /day of fish oil (SOFO)
(iv) 105 g /day of fish oil (FO)

Cows were grazed on a predominantly perennial ryegrass sward and the oils were fed in a concentrate mixture, which was offered at a rate of 3.0 kg/cow per day, after the morning milking. Sunflower oil (Trilby Trading Ltd., Drogheda, Ireland) was included directly as the oil and the fish oil was included as Omega-3 Supplement (UFAC UK Ltd., Waterwitch House, Exeter Road, Newmarket, Suffolk, UK CB8 8XR). This is a free flowing meal comprised of marine oil and an extracted oilseed meal and it contains 500 g/kg of oil. Concentrate supplements were sampled once weekly and analysed for DM, CP and ash content. The composition of the supplements is shown in Table 5. Grazing was on a rotational basis with cows being given access to a fresh paddock each 24h. Pre-grazing, two approximately 10 X 1.2m strips of grass were cut once weekly with an Agria mower (Agria-Werke GmbH, Mockmuhl, Germany) to 4 cm and sampled to determine grass available and grass quality. The pre-grazing yield of grass was (mean ± sd) 2.84 ± 0.841 t DM/ha. The composition of the grass was DM 181 ± 17.9 g/kg, CP 172 ± 27.3 g/kg DM, ADF 265 ± 16.1 g/kg DM, Ash 90 ± 13.4 g/kg DM and OMD 808 ± 23.0 g/kg OM.

*2.2 Milk yield and composition*

Cows were milked twice daily at 0730 and 1500 h and milk yield was recorded at each milking using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat, protein, and lactose) was determined once weekly from successive morning and evening samples by automated infrared absorption analysis using a MilkoScan 605 (Foss Electric, Hillerød, Denmark).

Individual cow milk samples were taken at the pm milking on day 0 (immediately before the supplements were introduced) and on days 14, 28 and 42 after the supplements were introduced. Milk fat was extracted and analysed for fatty acid composition as outlined by Coakley *et al*., (2007).

*2.3 Cow live weight and body condition score*

Individual cow live weight (LW) was measured once weekly after morning milking during each experimental week using electronic scales (Tru-Test Ltd., Auckland, New Zealand). Individual cow body condition score (BCS) (Lowman *et al*., 1976) on a scale of 0 (thinnest) to 5 (fattest) was determined once weekly in conjunction with the LW measurement. The LW change and BCS change were calculated as the difference between the values of LW and BCS, respectively from the first and last weeks of the study

***Statistical analysis***

A mixed model using PROC MIXED (SAS, 2005) was used to compare treatments for milk yield, milk constituent yield milk composition and milk fatty acid concentrations. The fixed factors included in the model were time (experimental week for milk yield, milk constituent yield and milk composition and sampling day for milk fatty acid concentrations) and treatment; cow was included as a repeated effect. The appropriate pre-experimental values were used as covariates. The LW and BCS changes were analysed using PROC GLM (SAS, 2005). The class variables included in the model were treatment and block. The following contrasts were made: supplemental oil versus no oil (SO, FO and FOSO versus P), oil source (SO versus FO) and the combined oil supplements versus the individual oils (SOFO versus SO and FO). Least square means are presented and effects were accepted as significant at $P < 0.05$.

***Results***

*3.1 Diet*

The grass offered was of good quality as indicated by the CP content of 172 g/kg DM and the OMD value of 808 g/kg OM. The average pre-grazing grass yield of 2.84 t DM/ha, however, was greater than would be recommended, which is about 1.8 t DM/ha for optimum grass quality (O'Donovan, 2000). The concentrate supplements had similar DM, CP and ash contents (Table 5), as would be anticipated, because almost 90 percent comprised unmolassed beet pulp and maize gluten feed in the same ratio.

*3.1 Cow Performance*

Cow performance on the different treatments is shown in Table 6. Type of oil had no significant effect on yield of milk, fat, protein or lactose or on milk composition. Including oil containing supplements increased the yield of milk ($P < 0.01$), protein ($P < 0.05$) and lactose ($P < 0.001$), decreased fat ($P < 0.05$) and protein ($p < 0.01$) concentrations and increased ($P < 0.01$) lactose concentration. Offering the two oils in combination decreased ($P < 0.05$) milk and lactose yields and increased ($P < 0.05$) protein concentration compared to offering the oils separately. There was no difference between treatments in BCS change but LW gain was greater ($P < 0.05$) on supplemented treatments than on P.

*3.2 Fatty acid composition of milk fat*

Offering oil containing supplements decreased the concentrations in milk of C6:0 ($P < 0.01$), C8:0 ($P < 0.01$), C10:0 ($P < 0.001$), C12:0 ($P < 0.01$) and C16:0 ($P < 0.001$) and increased the concentrations of C18:0 ($P < 0.05$), vaccenic acid ($P < 0.001$), C18:1 *cis 9* ($P < 0.001$), C18:2 *cis* 9, *cis* 12 ($P < 0.001$) and CLA ($P < 0.01$) (Table 7). There were differences between the sunflower oil and fish oil in their effects on some fatty acid concentrations. The concentrations of C4:0 ($P < 0.05$), vaccenic acid ($P < 0.01$) and CLA ($P < 0.05$) were greater on FO while the concentrations of C18:0 and C18:3 *cis* 9, *cis* 12, *cis* 15 were greater ($P < 0.01$) on SO. Supplementing with a mixture of the two oils compared with offering them separately decreased the concentrations of C4:0 ($P < 0.001$) and C6:0 ($P < 0.05$) and increased the concentrations of C18:0 ($P < 0.01$) and CLA ($P < 0.05$).

**Table 5** The ingredient and chemical composition (g/kg DM unless stated otherwise) of the concentrate supplements offered.

| | **Concentrate** | | |
|---|---|---|---|
| | **SO** | **SOFO** | **FO** |
| Unmolassed beet pulp | 688 | 661 | 700 |
| Maize gluten feed | 197 | 189 | 200 |
| Sunflower oil | 85 | 85 | |
| [1]Omega-3 supplement | | 35 | 70 |
| [2]Minerals/vitamins | 30 | 30 | 30 |
| | | | |
| DM | 927 ± 1.1 | 929 ± 1.2 | 924 ± 1.0 |
| CP | 109 ± 1.0 | 105 ± 1.3 | 113 ± 1.6 |
| Ash | 88 ± 1.8 | 89 ± 1.6 | 90 ± 4.4 |

[1]This is a mixture of marine oils and an extracted oilseed meal. It contains 500 g/kg of oil

[2]The mineral mixture contained di-calcium phosphate, limestone flour, salt and calcined magnesite plus the following amounts (g unless stated otherwise) of trace minerals and vitamins

| | | | |
|---|---|---|---|
| Manganese Oxide | 80 | Cobalt Sulphate | 10 |
| Copper Sulphate | 200 | Vitamin A | 8 MIU |
| Zinc Oxide | 125 | Vitamin $D_3$ | 2 MIU |
| Potassium Iodate | 18 | Vitamin E | 150,000 IU |
| Sodium Selenite(46% Se) | 2 | | |

**Table 6** Milk production and composition and LW and BCS changes on the different treatments

| | Treatments[1] | | | | | Contrast[2] | | |
|---|---|---|---|---|---|---|---|---|
| | P | SO | SOFO | FO | sem | 1 | 2 | 3 |
| Yield (kg/day) | 22.0 | 23.9 | 22.9 | 24.5 | 0.45 | <0.01 | 0.42 | <0.05 |
| Fat (g/day) | 830 | 845 | 805 | 829 | 31.7 | 0.91 | 0.69 | 0.33 |
| (g/kg) | 37.7 | 35.3 | 35.6 | 34.2 | 1.30 | <0.05 | 0.44 | 0.48 |
| Protein (g/day) | 755 | 809 | 775 | 810 | 15.8 | <0.05 | 0.97 | 0.08 |
| (g/kg) | 34.4 | 34.0 | 34.0 | 33.3 | 0.65 | <0.01 | 0.42 | <0.05 |
| Lactose (g/day) | 1001 | 1138 | 1063 | 1142 | 26.1 | <0.001 | 0.91 | <0.05 |
| (g/kg) | 45.5 | 47.6 | 46.2 | 46.8 | 0.51 | <0.01 | 0.18 | 0.07 |
| LW gain (kg/day) | 0.94 | 1.35 | 1.22 | 1.14 | 0.103 | <0.05 | 0.14 | 0.86 |
| BCS gain | 0.24 | 0.16 | 0.24 | 0.14 | 0.084 | 0.57 | 0.85 | 0.40 |

[1]P = pasture only; SO = pasture ad-lib plus 3 kg supplement supplying 255 g of sunflower oil; SOFO = pasture ad-lib plus 3 kg of supplement supplying 255 g of sunflower oil and 52.5 g of oil/day from the Omega-3 supplement; FO = pasture ad-lib plus 3 kg of supplement supplying 105 g of oil/day from the Omega-3 supplement

[2]1 = Pasture only diet vs. pasture plus added fat (P vs. SO, SOFO and FO), 2 = pasture plus sunflower oil diet vs. pasture plus fish oil diet (SO vs. FO), 3 = average of pasture plus sunflower oil diet and pasture plus fish oil diet vs. pasture plus sunflower and fish oil diet (SO and FO vs. SOFO)

**Table 7** Effect of treatment on the concentration of the main fatty acids in milk

| | Treatment[1] | | | | | Contrast[2] | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid (g/100g of Fatty Acid Methyl Esters) | P | SO | SOFO | FO | sem | 1 | 2 | 3 |
| C4:0 | 1.46 | 1.49 | 1.36 | 1.61 | 0.039 | 0.47 | <0.05 | <0.001 |
| C6:0 | 1.43 | 1.33 | 1.24 | 1.38 | 0.036 | <0.01 | 0.35 | <0.05 |
| C8:0 | 1.10 | 0.99 | 0.92 | 1.03 | 0.036 | <0.01 | 0.43 | 0.08 |
| C10:0 | 2.77 | 2.21 | 2.22 | 2.51 | 0.104 | <0.001 | 0.05 | 0.28 |
| C12:0 | 3.32 | 2.79 | 2.78 | 3.07 | 0.113 | <0.01 | 0.09 | 0.29 |
| C14:0 | 11.24 | 10.38 | 10.72 | 10.96 | 0.254 | 0.07 | 0.11 | 0.88 |
| C14:1c | 1.15 | 1.28 | 1.13 | 1.21 | 0.058 | 0.39 | 0.35 | 0.07 |
| C16:0 | 25.51 | 22.31 | 23.61 | 23.31 | 0.445 | <0.001 | 0.07 | 0.10 |
| C16:1c | 1.77 | 1.62 | 1.64 | 1.72 | 0.063 | 0.13 | 0.26 | 0.70 |
| C18:0 | 8.90 | 9.95 | 10.42 | 8.74 | 0.290 | <0.05 | <0.01 | <0.01 |
| C18:1 trans-9 + 18:1 trans-11[3] | 4.36 | 5.61 | 6.98 | 7.10 | 0.309 | <0.001 | <0.01 | 0.13 |
| C18:1 trans-13 + 18:1 cis-9[3] | 19.46 | 23.44 | 23.40 | 22.25 | 0.499 | <0.001 | 0.11 | 0.38 |
| C18:2 cis-9, cis-12 | 0.83 | 1.50 | 1.53 | 1.40 | 0.041 | <0.001 | 0.09 | 0.14 |
| C18:3 cis-9, cis-12, cis15 | 0.71 | 0.74 | 0.65 | 0.64 | 0.023 | 0.13 | <0.01 | 0.17 |
| C18:2 cis9, trans-11 CLA | 1.76 | 1.87 | 2.36 | 2.16 | 0.105 | <0.01 | <0.05 | <0.05 |

[1]P = pasture only; SO = pasture ad-lib plus 3 kg supplement supplying 255 g of sunflower oil; SOFO = pasture ad-lib plus 3 kg of supplement supplying 255 g of sunflower oil and 52.5 g of oil/day from the Omega-3 supplement; FO = pasture ad-lib plus 3 kg supplement supplying 105 g of oil/day from the Omega-3 supplement

[2] 1 = Pasture only diet vs. pasture plus added fat (P vs. SO, SOFO and FO), 2 = pasture plus sunflower oil diet vs. pasture plus fish oil diet (SO vs. FO), 3 = average of pasture plus sunflower oil diet and pasture plus fish oil diet vs. pasture plus sunflower and fish oil diet (SO and FO vs. SOFO)

[3]The peaks generated on the gas chromatograph for C18:1 *trans*-9 and C18:1 *trans*-11 vaccenic acid are very close and are thus difficult to separate accurately, so to avoid subjectivity on the division of the peaks, these 2 peaks are counted as one, with C18:1 *trans*-11 vaccenic acid being the major fatty acid present. This also occurred with C18:1 *trans*-13 and C18:1 *cis*-9 oleic acid, with C18:1 *cis*-9 oleic acid being the major fatty acid present.

***Conclusions***

This study confirms the previously reported relatively high concentrations of CLA and vaccenic acid in milk produced on pasture. It also demonstrates that the concentration of CLA can be increased further by 34 % by offering a supplement containing approximately 50 g of fish oil plus 255 g of sunflower oil and by 23 % by offering a supplement containing approximately 100 g of fish oil only per cow per day. The corresponding increases in vaccenic acid were 60 % and 63 %, respectively. The increase in the concentration of these fatty acids would appear to be associated more with fish oil rather than sunflower oil supplementation, and a combination of fish oil and sunflower oil are more effective than either oil alone. These improvements were achieved without any negative impacts on the yields of milk, fat or protein.

## 3.4 Experiment 4

**Introduction**

The benefits of grazing compared to silage feeding in relation to milk fatty acid composition and CLA content are well documented (Chilliard et al., 2001). Offer (2002) and Elgersma et al. (2003) found that beneficial fatty acids including CLA were higher in milk from grazed animals compared to those fed grass silage. Further they observed that zero-grazed cows were significantly lower than grazed cows in milk CLA production. A similar study led by Leiber et al. (2005) wherein the influence of altitude was also investigated among pasture (grazing), barn (zero-grazing) and silage fed cows reported that milk CLA concentrations were significantly higher in pasture compared to barn fed cows. Because of these interesting findings it is worth investigating the possible mechanisms underpinning the differences in milk CLA between cows grazing zero-grazing or fed grass silage. With this objective, an experiment was designed comparing diets of grazed grass (G), zero-grazed grass (ZG) and grass silage (GS) (harvested from the same perennial rye grass sward) to investigate the effect of the different forage types on the quality of milk fat. The specific objective was to systematically investigate the possible factors responsible for the differences in milk vaccenic acid and CLA contents.

***Materials and methods***

Six spring-calving rumen cannulated pluriparous (lactation number 2 to 5) Holstein Friesian dairy cows in early lactation (average milk yield – 30.4 ± 3.7 L/d; average days in milk – 76 ± 18 days; average bodyweight – 542 ± 34 kg) were balanced for milk yield and days in milk and assigned to two squares. Within a square the animals were randomly assigned to 3 treatments – G, ZG and GS in a 3×3 Latin square design with three 21 day periods. The experiment was carried out between June and August on a perennial ryegrass sward. All the experimental animals were grazing a perennial rye grass sward before the feeding experiment. Silage was harvested from the same perennial rye grass sward, field wilted for two hours and wrapped in plastic bales in May.

A grazing area of 1.7 ha consisted of two main grazing blocks. These were subdivided into two equal halves to offer grass for G or ZG animals. Paddock area utilized for the study was divided width wise in such a way that the G animals and ZG animals received the same quality of grass throughout the experiment. Individual intakes for the grazing animals were measured using a controlled release n-alkane marker (Alkane CRC, Captec (NZ) Ltd, Auckland, New Zealand) in each period. Indoor

animals were offered *ad-libitum* grass or silage. The intakes were adjusted to have at least 5-10 % of weigh-backs. Grass offered to the ZG animals was cut twice daily at 4 cm height from the ground with a motor Agria (Etesia UK Ltd., Warwick, UK). Offered and refused grass and silage samples were collected twice daily from the indoor fed cows during the experimental period and DM intakes calculated. A concentrate containing rolled barley (300 g/kg), citrus pulp (460 g/kg) soya bean meal (180 g/kg), soya bean oil (30 g/kg) and mineral/vitamin mix (30 g/kg) was offered to all cows at a rate of 3 kg/day before the evening milking. Milk yield was recorded daily. Milk sampling was done twice (AM and PM) on days 18 and 19 of each period. The morning and evening samples were pooled for each cow on each sampling day in proportion to the morning and evening milk yields. Milk samples from the two consecutive days were again pooled in proportion to the daily yields and divided into two aliquots. One aliquot was preserved with potassium dichromate and analyzed for protein, fat, and lactose using a Milkoscan 203 (Foss Electric DK-3400, Hillerod, Denmark). The second aliquot was stored at -20$^{o}$C until analyzed for milk fatty acid composition using gas chromatography.

***Statistical Analysis***

Feed FA data were analyzed using GLM procedure of SAS (version 9.1.3, SAS Institute Inc.). Dry matter intakes, LA and LNA intake, efficiency of milk RA production, milk yield, composition, rumen pH, VFA, ammonia concentrations and FA data from rumen, plasma and milk were analysed using the MIXED procedure of SAS (version 9.1, SAS Institute, Cary, NC) with treatments as the fixed effect; square, period and cow (square) as random effects. Differences between least square means were declared significant when $P$ value was < 0.05. Step-wise regression analysis was used to identify the variables that most influenced milk RA yield with multiple factors using STEP WISE procedure of SAS. The threshold value to keep a term in the model was $P \leq 0.09$.

***Results***

*Dry matter intake, Milk yield, composition and CLA yield*

The chemical composition of the forages and the principal fatty acids (as a % of total fatty acid methyl esters are presented in Table 8.

**Table 8** Chemical composition and the principal fatty acids in the forage component of diets

| | Diets[1] | | |
|---|---|---|---|
| | G | ZG | GS |
| DM % | 18.6 | 18.2 | 22.9 |
| CP % | 20.4 | 19.8 | 12.1 |
| Total fatty acids %[2] | 2.5 | 2.1 | 1.4 |
| NDF % | 41.5 | 45.4 | 50.8 |
| ADF% | 28.1 | 25.5 | 30.1 |
| Ash% | 12.9 | 9.4 | 7.4 |
| C16:0 | 14.9 | 15.5 | 17.2 |
| C18:0 | 1.7 | 1.8 | 2.4 |
| C18:1 | 2.6 | 3.0 | 4.3 |
| C18:2n-6 | 10.0 | 11.0 | 14.4 |
| C18:3n-3 | 57.7 | 54.6 | 47.1 |

[1]Diets were grazing (G), zero-grazing (ZG) and grass silage (GS) Concentrate fed had DM – 86.2 %; CP – 16.5 %; total fatty acid – 1.9% and Crude Fibre – 8.5 %

[2]Expressed as triglycerides

Grass DMI and total DMI were significantly different between G and GS with no difference between G and ZG diets (Table 9). Milk yield was significantly different across the three treatments. It was highest on G and lowest on GS. The mean milk yields on ZG and S were 18.3% and 34.6% lower, respectively, in comparison to G. There was no difference in lactose content between the treatments. Milk fat was significantly lower on GS compared to ZG. Milk protein was significantly lower on GS with no difference between G and ZG. Milk fat yield, protein yield and lactose yield were significantly different between the treatments with higher values recorded in G and lower values in GS.

**Table 9.** Dry matter intake, milk yield and milk composition

| | Diet[1] | | | | |
|---|---|---|---|---|---|
| | G | ZG | GS | *P* value | SEM |
| Forage DMI (kg/d) | 16.10$^{a}$ | 15.30$^{a}$ | 12.96$^{b}$ | 0.02 | 0.60 |
| Total DMI (kg/d) | 18.70$^{a}$ | 17.90$^{a}$ | 15.56$^{b}$ | 0.02 | 0.60 |
| Milk yield (L/d) | 24.62$^{a}$ | 20.12$^{b}$ | 16.11$^{c}$ | < 0.01 | 1.33 |
| Fat (%) | 3.78$^{ab}$ | 3.97$^{a}$ | 3.49$^{b}$ | 0.04 | 0.36 |
| Protein (%) | 3.37$^{a}$ | 3.22$^{a}$ | 2.81$^{b}$ | < 0.01 | 0.17 |
| Lactose (%) | 4.58 | 4.47 | 4.17 | NS | 0.15 |
| Fat yield (kg/d) | 0.93$^{a}$ | 0.79$^{b}$ | 0.58$^{c}$ | < 0.01 | 0.05 |
| Protein yield (kg/d) | 0.82$^{a}$ | 0.64$^{b}$ | 0.47$^{c}$ | < 0.01 | 0.04 |
| Lactose yield (Kg/d) | 1.13$^{a}$ | 0.90$^{b}$ | 0.70$^{c}$ | < 0.01 | 0.06 |
| RA[2] yield (g/d) | 18.99$^{a}$ | 10.77$^{b}$ | 2.81$^{c}$ | < 0.01 | 1.13 |

$^{abc}$ values in the same row not sharing a common superscript are significantly different

[1]Diets were grazing (G), zero-grazing (ZG) and grass silage (GS)

[2]RA = CLA

*Milk fatty acid composition*

The concentrations of the major saturated fatty acids (14:0 and 16:0) were lower in G and ZG compared to GS. There was no significant difference in the concentrations of

short-chain FA (excepting 6:0 and 8:0) between treatments. The concentrations of 18:1, 18:2 and 18:3 were significantly higher on G and ZG than on GS

**Table 10** Effect of treatments on the main milk fatty acid composition (g/100g fatty acid methyl esters)

| | Diets[1] | | | | |
|---|---|---|---|---|---|
| Fatty acids[2] | G | ZG | GS | *P* value | SEM |
| 4:0 | 3.63 | 3.87 | 3.94 | NS | 0.20 |
| 6:0 | 2.34[b] | 2.57[a] | 2.57[a] | 0.03 | 0.12 |
| 8:0 | 1.34[b] | 1.45[a] | 1.39[ab] | 0.08 | 0.09 |
| 10:0 | 2.76 | 3.02 | 2.95 | NS | 0.28 |
| 12:0 | 2.96 | 3.16 | 3.29 | NS | 0.29 |
| 14:0 | 9.8[b] | 10.4[ab] | 11.2[a] | 0.02 | 0.37 |
| *c*9-14:1 | 0.94[ab] | 0.89[b] | 1.16[a] | 0.09 | 0.11 |
| 15:0 | 1.11[b] | 1.10[b] | 1.34[a] | < 0.01 | 0.03 |
| 16:0 | 23.7[c] | 26.3[b] | 37.9[a] | < 0.01 | 1.15 |
| 16:1 | 1.51[b] | 1.42[b] | 1.94[a] | < 0.01 | 0.13 |
| 18:0 | 10.1[a] | 10.6[a] | 7.3[b] | < 0.01 | 0.42 |
| *c*-18:1 | 19.53[a] | 17.55[b] | 13.92[c] | < 0.01 | 1.16 |
| 18:2n-6 | 1.08[a] | 1.03[a] | 0.82[b] | < 0.01 | 0.16 |
| 18:3n-3 | 0.68[b] | 0.82[a] | 0.34[c] | < 0.01 | 0.13 |
| 20:0 | 0.12[b] | 0.14[a] | 0.14[a] | 0.03 | 0.01 |

[abc] Values in the same row not sharing a common superscript are significantly different ($P < 0.05$).

[1]Diets: G = grazing; ZG = zero-grazing; GS = grass silage.

[2]FAME = fatty acid methyl esters (expressed as a percentage of total FAME; *c* = *cis*; *t* = *trans*; CLA = conjugated linoleic acid; SFA = saturated fatty acid; SCFA = short-chain fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

*Rumen, plasma and milk vaccenic acid and CLA*

The concentrations of vaccenic acid in rumen, plasma and milk followed the same trend with its concentration being significantly higher in G than ZG which in turn was higher than in GS (Table 11). The concentration of CLA in rumen and plasma, while being significantly different between treatments, was extremely low. Treatment G had a significantly higher concentration of CLA in milk than ZG which in turn had a significantly higher concentration than GS.

Among the total CLA isomers in milk *c*9*t*11-CLA was the predominant CLA isomer accounting for 86%, 83% and 77% in G, ZG and GS, respectively.

**Table 11** Effect of treatments on the concentrations of vaccenic and CLA in rumen, plasma and milk and the ratio of C14:1/C14:0 in milk

| | **Diets**[1] | | | | |
|---|---|---|---|---|---|
| % total FAME[2] | G | ZG | GS | *P* value | SEM |
| **Rumen** | | | | | |
| Vaccenic acid | 12.30[a] | 9.31[b] | 4.21[c] | < 0.01 | 0.66 |
| CLA | 0.09[b] | 0.09[b] | 0.13[a] | 0.04 | 0.03 |
| **Plasma** | | | | | |
| Vaccenic acid | 2.18[a] | 1.47[b] | 0.66[c] | < 0.01 | 0.20 |
| CLA | 0.17[a] | 0.14[ab] | 0.11[b] | 0.01 | 0.01 |
| **Milk** | | | | | |
| Vaccenic acid | 4.73[a] | 3.49[b] | 0.99[c] | < 0.01 | 0.30 |
| CLA | 2.07[a] | 1.38[b] | 0.54[c] | < 0.01 | 0.17 |
| C14:1/C14:0 | 0.10 | 0.09 | 0.10 | NS | 0.01 |

[abc] Values in the same row not sharing a common superscript are significantly different ($P < 0.05$).
[1] Diets: G = grazing; ZG = zero-grazing; GS = grass silage.
[2] FAME = fatty acid methyl esters

Mammary desaturase activity calculated based on the ratio of milk 14:1/14:0 was not different between the treatments (Table 11). Although desaturase activity can be determined using ratios of 4 different pairs of FA, the ratio 14:1/14:0 has been reported to give a true picture of mammary desaturase activity since 14:1 is a de novo synthesized FA by the mammary tissue.

Step-wise regression analysis using the variables - substrate intake (C18:2 *cis-9, cis-12,* C18:3 *cis-9, cis-12, cis-15*), ruminal vaccenic acid, plasma vaccenic acid, wet and dry rumen fill, rumen pH, and milk desaturase activity revealed that 95.3% of the variability in milk CLA yield was determined by the differences in plasma vaccenic acid concentrations (Table 12). The variables subsequently selected in the model included C18:3 *cis-9, cis-12, cis15* intake and C18:2 *cis-9, cis-12* intake, although their contribution in improving the model $R^2$ was very small. Milk desaturase activity did not enter into the model even at a significance level of 0.2 suggesting that mammary desaturase activity was not associated with differences in milk CLA and desaturase activity was not influenced by the treatments in the current study.

**Table12** Step-wise regression analysis of a group of variables* with milk CLA yield (g/d) (n = 17; $P = 0.09$)

| Variable | Partial $R^2$ | Model $R^2$ | F value | *P* value |
|---|---|---|---|---|
| Plasma vaccenic acid (a) | 0.9533 | 0.9533 | 265.63 | <0.01 |
| Intake *cis-9, cis-12, cis-15* (b) | 0.0319 | 0.9853 | 25.97 | <0.01 |
| Intake C18:2 *cis-9, cis-12* (c) | 0.0063 | 0.9915 | 8.17 | 0.02 |
| Equation | CLA yield g/d = 15.77 + 7.38a + 0.14b – 0.67c | | | |

*Independent variables considered for the model include – intake 18:2n-6, 18:3n-3, rumen vaccenic acid (VA), plasma VA, wet rumen fill, dry rumen fill, rumen pH and milk desaturase index.

*Substrate intake and efficiency of milk CLA production*

The intakes of C18:2 *cis-9, cis-12* and *cis-9, cis-12, cis-15* were significantly higher in G compared to ZG. Total substrate intake was also significantly different across the treatments (Table 13). Significantly lower intakes of C18:2 *cis-9, cis-12* and *cis-9, cis-12, cis-15* in GS fed cows compared to other treatments could be explained by the lower DMI as well as the lower proportion of *cis-9, cis-12, cis-15* in GS. Lower proportion of *cis-9, cis-12, cis-15* in GS relative to G and ZG could be due to oxidation of PUFA during wilting and when grass is ensiled. CLA yield per 100 g total substrate intake (efficiency) was significantly different ($P < 0.01$; SEM – 0.17) across the treatments with higher values recorded in G and lower values in GS. Efficiency of milk CLA production (g CLA/100g substrate) in G (2.23) was significantly higher than that in ZG (1.50) or GS (0.62) suggesting that G cows were more efficient than ZG or GS cows because they were able to produce more CLA per 100 g of total substrate intake. These differences in the efficiency of milk CLA production could be due to a combination of factors regulating ruminal vaccenic acid production and its supply to the mammary tissue.

**Table 13** Substrate intake (from total DMI) and efficiency of milk CLA production

| | Diets[1] | | | | |
|---|---|---|---|---|---|
| | G | ZG | GS | *P* value | SEM |
| C18:2 *cis-9, cis-12* intake g/d | 91.80[a] | 76.71[b] | 50.54[c] | < 0.01 | 2.90 |
| *cis-9, cis-12, cis-15* intake g/d | 341.49[a] | 249.91[b] | 113.32[c] | < 0.01 | 17.24 |
| Total substrate intake[2] g/d | 433.28[a] | 326.62[b] | 163.86[c] | < 0.01 | 17.89 |

[abc] values in the same row not sharing a common superscript are significantly different ($P < 0.05$).
[1]G = grazing; ZG = zero-grazed grass; GS = grass silage; HS = herbage snips representing the grass consumed by G cows; GC = grass cuts representing the grass offered to the G cows.
[2]Total substrate = sum of C18:2 *cis-9, cis-12* and *cis-9, cis-12, cis-15* in diet.

***Conclusions***

From the above findings, it could be concluded that although the differences in substrate intake appear to contribute largely to the differences in milk CLA response between the treatments, there could be differences in the rumen environment and or digesta kinetics between the treatments and possibly diet and animal genotype interaction that is contributing to the added increased efficiency of G cows on milk CLA production. Overall, findings suggest that 95% of the variability in milk CLA yield in the current study was influenced by the differences in plasma vaccenic acid content. The differences in the concentrations of plasma vaccenic acid between the treatments could be attributed to the differences in *cis-9, cis-12, cis-15* intake, dietary influence on rumen environment and possibly animal genetics.

## 3.5 Experiment 5

***Introduction***

Many dietary factors which affect CLA concentrations in milk fat have been researched and described over the last decade. In Ireland our pasture based system of milk production results in milk with naturally higher concentrations of CLA compared with indoor systems of production and studies presented here and elsewhere have demonstrated that these naturally high concentrations can be increased further by

supplementation with oils. Furthermore the CLA content of milk fat from individual cows is variable ranging from approximately 5 mg/g of fat to over 30 mg/g of fat. Possible reasons for this variation are the differential genetic expression of delta-9 desaturase, and/or the capacity of different cows to produce different amounts of vaccenic acid in their rumens. By exploiting this natural variation in CLA production in combination with fish oil supplementation at pasture, which has been shown to increase milk CLA concentrations (Murphy et al., 2008) it may be possible to achieve exceptionally high CLA concentrations in milk fat from selected cows.
The objective of this experiment was to evaluate the response of high and low CLA cows to a supplement that is known to enhance CLA concentrations.

***Materials and Methods***

Forty autumn calving cows from a total herd of 70 were used in the study. A milk sample for CLA analysis was taken from the cows in early April when they were all on a diet of grazed grass. The cows were ranked from highest to lowest on the basis of their CLA concentrations. The highest 20 and lowest 20 were allocated to a high CLA and low CLA group, respectively. Within these groups cows were then blocked into the following two treatments on the basis of CLA concentration and milk yield:

1. Pasture ad-libitum (Control)
2. Pasture ad-libitum plus 3.0 kg/day of supplement containing 105g/day of fish oil (Omega 3 supplement – UFAC, UK Ltd.)

The Omega-3 supplement included was the same as that used in Experiment 3.3. The composition (g/kg) of the concentrate supplement fed was: citrus pulp 700, maize gluten feed 200, omega-3 supplement 70 and minerals vitamins 30. Treatments were put in place on April 30 and cows remained on treatments until June 12 (43 days).

*Milk yield and composition*

Cows were milked twice daily at 0730 and 1500 h and milk yield was recorded at each milking using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat, protein, and lactose) was determined once weekly from successive morning and evening samples by automated infrared absorption analysis using a MilkoScan 605 (Foss Electric, Hillerød, Denmark).
Individual cow milk samples were taken at the pm milking in weeks 1, 3 and 7 (May 1, May 15 and June 12). Milk fat was extracted and analysed for fatty acid composition as outlined by Coakley *et al*., (2007).

*Cow live weight and body condition score*

Individual cow live weight (LW) was measured once weekly after morning milking during each experimental week using electronic scales (Tru-Test Ltd., Auckland, New Zealand). Individual cow body condition score (BCS) (Lowman *et al*., 1976) on a scale of 0 (thinnest) to 5 (fattest) was determined once weekly in conjunction with the LW measurement. The LW change and BCS change were calculated as the difference between the values of LW and BCS, respectively from the first and last weeks of the study

*Grass yield and samples*

Grass was sampled once-weekly pre and post grazing to determine grass available and grass consumed. Pre grazing samples were analysed for dry matter (DM), crude

protein (CP), ash, neutral detergent fibre, organic matter digestibility and fatty acid composition.

*Supplement samples*
The supplement offered was sampled once weekly and analysed for DM, CP, ash, crude fibre, oil and fatty acid composition.

***Statistical analysis***
A mixed model using PROC MIXED (SAS, 2005) was used to compare treatments for milk yield, milk constituent yield milk composition and milk fatty acid concentrations. The fixed factors included in the model were time (experimental week) main treatment, sub treatment and their interactions; block was treated as a random effect and an autoregressive covariance structure was used. The LW and BCS changes were analysed using PROC GLM (SAS, 2005). The class variables included in the model were main treatment, sub treatment and block.

***Results***
Pre-grazing pasture yield was 2.11 ± 1.09 t DM/ha and pre and post-grazing sward heights were 123 ± 27.0 mm and 44 ± 5.3 mm, respectively. The composition of the grass and concentrate supplement offered is shown in Table 14. Both feeds had composition as expected with the grass being of high quality as indicated by the organic matter digestibility.

**Table 14** The composition (g/kg DM) of the grass and concentrate supplement consumed during the study. Values are presented as mean ± sd.

| | **Grass** | **Concentrate** |
|---|---|---|
| DM | 170 ± 35.5 | 937 ± 4.0 |
| CP | 228 ± 38.8 | 102 ± 2.6 |
| NDF | 419 ± 34.1 | |
| CF | | 120 ± 1.0 |
| Ash | 110 ± 16.0 | 98 ± 1.4 |
| Ether Extract | 34 ± 4.4 | 70 ± 4.0 |
| OMD | 829 ± 10.3 | |

There was no difference between the low and high CLA treatments in any of the production variables measured (Table 15). Offering the supplement resulted in greater milk yield ($P<0.001$), fat yield ($P<0.05$), protein yield ($P<0.001$), lactose yield ($P<0.001$) and protein concentration ($P<0.05$).Nether the High or Low CLA treatment or the offering of supplement affected LW gain or BCS change.

**Table 15** Milk production and composition, LW and LW and BCS changes on the main and sub treatments

| | **Main Treatment (M)** | | **Sub Treatment (S)** | | | **Significance of Effects** | | |
|---|---|---|---|---|---|---|---|---|
| | Low CLA | High CLA | No Suppl. | Plus Suppl. | sem | M | S | M*S |
| Yield (kg/day) | 19.2 | 19.6 | 18.4 | 20.4 | 0.30 | | <0.001 | |
| Fat (g/day) | 702 | 704 | 682 | 723 | 13.2 | | <0.05 | |
| (g/kg) | 36.6 | 36.3 | 37.1 | 35.7 | 0.68 | | | |
| Protein (g/day) | 661 | 660 | 635 | 686 | 10.0 | | <0.001 | |
| (g/kg) | 34.4 | 34.0 | 34.5 | 33.9 | 0.21 | | <0.05 | |
| Lactose (g/day) | 856 | 883 | 820 | 919 | 14.6 | | <0.001 | |
| (g/kg) | 44.7 | 44.9 | 44.6 | 45.1 | 0.22 | | | |
| Average LW (kg) | 589.2 | 584.0 | 585.7 | 587.5 | 3.06 | | | |
| LW gain (kg/day) | 0.92 | 0.74 | 0.81 | 0.85 | 0.08 | | | |
| BCS change | -0.01 | -0.04 | -0.01 | -0.04 | 0.029 | | | |

Selecting cows for high and low CLA had no significant effect on most of the fatty acids but as would be expected resulted in greater CLA ($P<0.05$) in the High treatment (Table 16). The High CLA group also had greater concentrations of C18:1 and C18:2. Including the supplement resulted in a greater concentration of vaccenic acid ($P<0.01$) and a numerically greater concentration of CLA ($P=0.12$). Offering supplement increased C18:2 ($P<0.001$) and decreased C18:3 ($P<0.001$). The C14:1/C14:0 (a proxy for mammary desaturase activity) was greater in the High than the Low CLA treatments indicating that desaturase activity probably contributed to the higher CLA content in the High treatment. There was no significant interaction between the main and sub treatments for CLA indicating that the High and Low treatment groups did not respond differently to a supplement designed to increase milk CLA content.

**Conclusions**

Cows grouped on the basis of their CLA content did not differ in their production characteristics. Cows selected for their High milk CLA content had a greater C14:1/C14:0 ratio indicating that greater mammary desaturase activity may be a contributory factor in their higher CLA. Groups of cows selected for their high and low CLA concentrations when on the same diet, did not respond differently when supplemented with fish oil which is known to increase milk CLA content. Therefore selecting high CLA cows and supplementing with a CLA-promoting diet will not result in the production of milk with extremely high CLA concentrations.

**Table 16** Effect of main and sub treatments on the concentration of the main fatty acids in milk

| | Main Treatment | | Sub Treatment | | | Significance of Effects | | |
|---|---|---|---|---|---|---|---|---|
| | Low CLA | High CLA | No Suppl. | Plus Suppl. | sem | M | S | M*S |
| C4:0 | 3.58 | 3.50 | 3.66 | 3.43 | 0.105 | | | |
| C6:0 | 1.77 | 1.71 | 1.75 | 1.73 | 0.032 | | | |
| C8:0 | 1.13 | 1.09 | 1.12 | 1.10 | 0.026 | | | |
| C10:0 | 2.52 | 2.38 | 2.51 | 2.39 | 0.073 | | | |
| C12:0 | 2.93 | 2.80 | 2.98 | 2.76 | 0.091 | | | |
| C14:0 | 9.86 | 9.84 | 9.99 | 9.71 | 0.159 | | | |
| C14:1c | 1.35 | 1.47 | 1.43 | 1.39 | 0.049 | | | |
| C16:0 | 23.34 | 24.05 | 23.95 | 23.44 | 0.333 | | | |
| C16:1c | 1.40 | 1.49 | 1.48 | 1.42 | 0.053 | | | <0.001 |
| C18:0 | 8.29 | 8.05 | 8.27 | 8.07 | 0.213 | | | |
| C18:1 trans-9 + 18:1 trans-11[2] | 5.48 | 5.24 | 4.85 | 5.84 | 0.242 | | <0.01 | |
| C18:1 trans-13 + 18:1 cis-9[2] | 20.7 | 21.7 | 21.3 | 21.1 | 0.345 | <0.05 | | |
| C18:2 cis-9, cis-12 | 0.97 | 1.08 | 0.90 | 1.16 | 0.038 | <0.05 | <0.001 | |
| C18:3 cis-9, cis-12, cis15 | 0.73 | 0.75 | 0.80 | 0.69 | 0.023 | | <0.001 | |
| C18:2 cis9, trans-11 CLA | 1.93 | 2.24 | 1.97 | 2.21 | 0.106 | <0.05 | | |
| C14:1/C14:0 ratio | 0.136 | 0.149 | 0.142 | 0.143 | 0.0052 | <0.05 | | |

[2]The peaks generated on the gas chromatograph for C18:1 *trans*-9 and C18:1 *trans*-11 vaccenic acid are very close and are thus difficult to separate accurately, so to avoid subjectivity on the division of the peaks, these 2 peaks are counted as one, with C18:1 *trans*-11 vaccenic acid being the major fatty acid present. This also occurred with C18:1 *trans*-13 and C18:1 *cis*-9 oleic acid, with C18:1 *cis*-9 oleic acid being the major fatty acid present.

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