Research Article

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Conjugated linoleic acid isomer concentrations in milk from high- and low-input management dairy systems

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Abstract

BACKGROUND: Different conjugated linoleic acid (CLA) isomers are known to have contrasting physiology or health effects and there is growing evidence that the profile of natural isomers in milk is influenced by the production system. This survey is the first to compare feeding regimes and concentrations of 14 CLA isomers in milk from three production systems in the UK.

RESULTS: Total CLA and seven isomers (including C18 : 2 c9t11 which comprised >80% of total) were significantly higher in milk from both organically certified and non-certified low input (LI) systems compared with milk from conventional high input farms. Sampling date also affected concentrations of total CLA and nine isomers; being lowest in March and highest in August. Seasonal differences were greater in milk from LI herds, thought to be due to changes in herbage and/or stage of lactation. Multivariate analysis showed a strong positive relationship between several CLA isomers and increasing levels of fresh forage in the diet.

CONCLUSIONS: These results add to the evidence on how management adjustment may improve the profile of CLA isomers in milk fat, although animal or human intervention studies are required to identify the effects of consuming milk with different CLA levels and isomer profiles on human health.

Keywords: CLA isomers; milk; organic; low input

INTRODUCTION

Dietary intake of conjugated linoleic acid (CLA) has been linked to physiological and health impacts in both animals and humans.[1–3] Positive health impacts were mainly extrapolated from cell culture and animal studies and included enhanced immune function and reduced risk of cancer, diabetes, atherosclerosis and obesity.[2,4,5] However, many human dietary intervention studies (most of which involved dietary supplementation with synthetic CLA) showed no significant or only minor impacts on health.[2,6] In contrast, a recent cohort study linked lower levels of eczema in infants from families consuming organic milk to a higher milk-related CLA intake, since CLA levels in organic milk were shown to be significantly higher than levels in conventional milk.[7–10]

Levels of CLA in milk and dairy products are of particular nutritional interest, since the main food sources of CLA in Western diets are dairy and meat products from ruminant animals, with dairy products contributing up to 70% of total CLA intake.[2,11] However, individual isomers of CLA are known to have contrasting physiological activities and the proportions of the two main CLA isomers linked to these health impacts were shown to differ significantly between synthetic and naturally occurring CLA in milk.[6,8,12,13] For example, the *cis*-9, *trans*-11 CLA isomer (c9t11, which was shown to account for more than 75% of CLA in milk) was most effective in reducing cancer risks in rats,[14,15] while the *trans*-10, *cis*-12 isomer (t10c12, which accounts for less than 1% of CLA in milk, but more than 30% in most synthetic CLA preparations) was more effective in reducing body fat in mice than the *cis*-9, *trans*-11 isomer.[16] Increasing the intake of the *trans*-10, *cis*-12 isomer in dairy cows via synthetic CLA preparation was also shown to reduce milk production, milk fat and lactose levels and increase somatic cell counts.[13] The same isomer was also linked to potential negative human health impacts.[17] Recent studies showed that the *trans*-9, *trans*-11 and *cis*-9, *trans*-11 isomers differ with respect to their cytotoxic activity against NCI-N87 gastric cancer cells.[18] Human intervention studies based on synthetic CLA supplements may therefore not allow accurate conclusions on the physiological/health impacts of CLA in milk and other ruminant fats.

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The origin of individual CLA isomers found in milk is also known to differ. A large proportion of the most abundant CLA isomer (c9t11) is thought to be generated endogenously in the mammary gland from vaccenic acid (VA; C18 : 1 t11)[19] which is produced in the rumen. In contrast, the *trans*-7, *cis*-9 isomer (t7c9) (the next most abundant isomer found in milk) originates solely from endogenous synthesis (from C18 : 1 t7 in this case).[20] All other CLA isomers found in milk or meat are also thought to be produced in the rumen by microbial biohydrogenation of unsaturated FA taken up with feed stuffs.[2,4,19,21,22] It is therefore likely that changes in management, especially feeding regimes, will have contrasting effects on the levels of different CLA isomers.

Previous comparisons have shown that milk from organic and other low-input production systems can have higher levels of nutritionally desirable fatty acids.[7,8,23] However, these studies focussed only on comparing total CLA levels and/or levels of CLA isomers c9t11 and t10 c12.[7,8] Some studies have investigated CLA isomer profiles in milk and assessed the impact of dairy production system-related factors, including feeding regimes, location and composition of grazing swards, stage of lactation, genotype, time of the year and/or diet supplementation with vegetable oils.[12,24–27] Only one previous study,[25] which focussed on grazing-based alpine milk production systems in Switzerland, compared CLA-isomer profiles in different low-input (organic and integrated) dairy production systems, but did not include high-input conventional production systems in the comparisons. However, this study identified differences in the profile of CLA isomers, despite relatively similar feeding regimes being used in the different production systems. The findings (1) of differences in biological activities between CLA isomers and (2) that different sward compositions and oil-seed feed supplements result in significant changes to CLA isomer profiles suggest that a wider range of CLA isomers needs to be assessed in studies comparing the milk from different production systems.[24,28] Such data will be essential to allow the design of dietary intervention and cohort studies which compare health impacts of CLA isomer profiles in milk from different production systems more accurately and impacts of 'natural' CLA in ruminant fat-based CLA intake with synthetic CLA supplementation approaches.

The aim of this study was to quantify both seasonal and management influences on CLA content and fatty acids associated with CLA synthesis and secretion into milk by:

(1) quantifying differences in the milk fat levels of 14 different CLA isomers between certified-organic 'low input' (O-LI) non-certified 'low input' (NO-LI) and standard 'high input' (HI) conventional production systems in South Wales. This will test the hypothesis that production systems significantly affect CLA-profiles in milk produced in regions with long grazing periods in the British Isles
(2) identifying the effect of sampling date and interactions between management systems and sampling date on the levels of different CLA isomers in milk fat and the proportion of different isomers in the total CLA content of milk fat. This will test the hypothesis that the level of contrast between CLA profiles of milk from different production systems changes throughout the year
(3) identifying the effect (and relative contribution) of individual production system components (e.g. diet composition, vitamin supplementation, breed, herd health related parameters) of dairy production systems on the levels of different CLA isomers in milk fat. This will test the hypothesis that contrasting CLA profiles are caused by differences in specific production system components (in particular feed composition) between production systems

The profiles of other fatty acids and antioxidants in milk from these systems has been studied and reported previously.[8]

MATERIALS AND METHODS

Farms used and design of the milk survey

Forty-six milk samples were collected from the bulk tanks of 13 commercial farms in Wales categorised into three different systems of production.

Conventional, high input

Five farms were selected which represented common conventional production and feeding systems in South Wales. All five farms used cereal or cereal by-product-based concentrate supplementation, grass silage as winter forage and synthetic vitamin A and E supplements. The high-input (HI) farms used predominantly rye grass or mixed grass swards, which were fertilised with mineral N and water-soluble P fertilisers according to standard practices in the geographical area and/or soil analyses. The HI group did not include farms with extremely high input/output system (e.g. farms which use more than 50% of the diet coming from concentrates, regularly milk three times per day, house animals throughout their lactation and/or achieve average lactation yields of $>10\,000$ L per cow per annum).

Organic certified 'low input'

Five farms were selected which represented common organic production and feeding systems in South Wales. These farms used no or low levels of cereal or cereal by-product-based concentrate supplementation, block calved in spring, grazed cows throughout the lactation and did not use synthetic vitamin A and E supplements. In accordance with organic certification rules, the organic, low-input (O-LI) farms used mixed grass–clover swards and did not apply mineral N or water-soluble P fertilisers. Where appropriate, on the basis of soil analyses, finely ground rock phosphate fertilisers were applied.

Non-organically certified 'low input'

Three farms were selected which represented a low-cost, 'low-input' New Zealand-type dairying system developed by the 'Grasshoppers' farmers association for the environmental conditions in south-western Wales. All farms used no or low levels of cereal or cereal by-product-based energy concentrate supplementation and block calved in the spring, grazed cows throughout the lactation and did not use synthetic vitamin A and E supplements. Non-organically certified 'low-input' (NO-LI) farms selected used mixed grass–clover swards, but applied up to 120 kg N ha^{-1} $year^{-1}$ of mineral N and water-soluble P fertiliser at levels recommended by soil analyses.

The farming systems selected have previously been described in detail.[8]

Extraction and analysis for fatty acids

Milk samples were taken in August and October in 2004, March and May 2005, frozen immediately after collection from the bulk tank and kept at $-20\,^{\circ}C$ before being analysed. They were warmed to

about 20 °C, centrifuged at 5000 × *g* for 30 min, and the separated creams were churned at approximately 5 °C. After the resulting molten butter had been filtered through a hydrophobic filter (1PS folded filter; Whatman, Bottmingen, Switzerland), the pure milk fat was collected and stored at −20 °C until analysis.

After dissolution of the pure milk fat in hexane, the glycerides were *trans*-esterified to the corresponding methyl esters of fatty acids with a solution of potassium hydroxide in methanol (ISO standard 15 884).[29]

Fatty acid composition was analysed by high-resolution gas chromatography (Agilent 6890; Agilent, Santa Clara, CA, USA) with flame ionisation detection according to Collomb and Bühler.[30] The fatty acids were separated on a capillary column CP-Sil 88 (100 m × 0.25 mm i.d. × 0.20 µm; Varian BV, Middleburg, Netherlands) and quantified using nonanoic acid as an internal standard. The results are expressed as g fatty acid per 100 g fat. The pure methyl esters of fatty acids, including CLA, were obtained from Matreya Inc., Pleasant Gap, PA, USA.

CLA isomers were analysed by silver ion (Ag^+) HPLC (Agilent LC 1100) equipped with a photodiode array detector (234 nm)) using three ChromSpher Lipid columns in series (stainless steel, 250 × 4.6 mm, 5 µm particle size; Chrompack, Middleburg, Netherlands) according to Collomb *et al*.[24] The solvent consisted of UV-grade hexane with 0.1% acetonitrile and 0.5% ethyl ether (flow rate 1 mL min^{-1}), prepared fresh daily. The injection volume was 10 µL, corresponding to <250 µg lipid. The HPLC areas for t7c9 (t = *trans*, c = *cis*) + t8c10 + c9t11 were added and used in the calculation of the peak area of the three isomers from the GC chromatogram. The results were expressed as absolute values as mg g^{-1} fat.

Recording the parameters for the farm production system

Details were recorded from each farm at each milk sampling date, including (1) numbers of lactating cows and heifers, (2) milk composition analysis as reported by milk purchasers (fat and protein content along with somatic cell count), (3) recent calvings, (4) veterinary treatments (including those relating to mastitis), (5) current feed and supplement use (including information on whether cows had access to pasture during the day and at night). Estimated grazing intakes were calculated for each herd, by difference, with total dry matter intakes estimated from average milk yields and assumed live weight (LW) (based on breed composition of the herds) (DMI = 0.025 LW + 0.125 milk yield). The shortfall between feeds specified by producers and total intakes were assumed to represent grazing intake, in situations when cows had access to pasture.

Cow breeds and crosses in each herd were identified and used to estimate the proportion of genes from breeds other than Holstein Friesian. Jersey was the main breed used for crosses, but herds also had crosses with Brown Swiss, Danish Red and/or Swedish Red.

A detailed description/comparison of production data and fatty acid composition of milk collected for the different systems and geographic regions was published previously.[8]

Statistical analysis

Univariate analyses

Linear mixed-effects models[31] were used to investigate differences in milk quality parameters under the different systems (HI, O-LI and NO-LI). Mixed-effects models use two types of explanatory variables: (1) fixed effects, which affect the mean of the response variable; and (2) random effects, which affect the variance of the response and can cope with an incomplete block design caused by missing samples.[32] In these analyses, farm identifier was used as a random effect. All proportion data were arcsine-transformed prior to statistical analysis, but means presented were calculated from non-transformed data. Pairwise comparisons of means were carried out, where appropriate, using the Tukey honest significant difference test, following the recommendation of Crawley.[33]

Multivariate analyses

Three indices were generated to represent diet, health and breed, for use as explanatory variables of all the milk composition data. A principal components analysis (PCA) was carried out using the proportions of fresh forage, conserved forage and concentrate to generate the diet index. Axis 1 PCA sample scores were used as the index, with high concentrates and silage inputs having the highest values and those dominated by fresh grass the lowest. The health index was a sum of the mastitis and other health treatments per milking cow, whilst the breed index was the proportion of genes from breed other than Holstein Friesian in the herd (see above). To investigate the relationship between the three indices and the distribution of individual CLA isomers, the whole data sets (irrespective of production system or sampling date) were used in a multivariate redundancy analysis (RDA).[33] In addition, an RDA was carried out using three diet components (proportions of fresh forage, conserved forage and concentrates) to investigate the relationship between diet composition and the CLA isomers.

The PCA and RDA analyses were carried out using the CANOCO package[34] whilst the *R* statistical environment[33] was used for all other analyses.

RESULTS AND DISCUSSION

The study reported here is the first to compare the levels of a wider range of 14 CLA isomers in milk from organic, low-input and high-input dairy production systems in the UK, and how these vary throughout the seasons. A detailed description of production system parameters used in the different farming systems included in this study has been reported previously[8] together with data on the fatty acid and antioxidant concentrations in the milk from these systems and are not described in detail here. However, since differences in the levels of total CLA and individual CLA isomers in milk fat between production systems and sampling dates were mainly related to differences in feeding regimes (see results from multivariate analyses below), a summary of the feeding regimes used in the three production systems is given below with details of select management factors shown in Table 1.

The two LI groups of farms grazed cows throughout the lactation period and used no or very low levels of concentrate supplementation or conserved forage (<5% of dry matter intake) and used a spring block calving system. The HI group of farms calved all year round, used concentrate supplementation and switched from grazing to grass–clover silage-based diets during the winter period (October and March sampling dates). Feeding regimes under the two LI sets of farms were similar in many respects and their comparison adds a valuable insight into the influences of subtle variations in management, as reported in the comparison of milk fat composition between organic and integrated farming systems in the Swiss Alps.[25]

Effect of production systems on CLA isomers in milk fat

The study confirmed that CLA isomer 18:2 c9t11 accounts for more than 80% of CLA found in milk fat. However, it also confirmed

Table 1. Differences in production system parameters recorded (or calculated) between high input conventional (HI), organically certified (O-LI) and non-organic (NO-LI) low input farms

	Production system			ANOVA results (*P* value)		
Parameter recorded	HI	O-LI	NO-LI	PS[†]	SD[†]	PSxSD[‡]
Herd and milk quality						
Herd size (milking cows)	316[a]	230[b]	394[a]	NS	**	***
Genotype index[§]	0.00[c]	0.13[b]	0.28[a]	***		
% primiparous cows	22[b]	31[a]	34[a]	*	NS	0.07
% cows in early lactation	28	50	40	**	***	***
Daily milk yield (L)	25[a]	17[b]	17[b]	***	***	NS
Fat content (g kg^{-1})	41[b]	45[a]	46[a]	**	***	0.08
Protein content (g kg^{-1})	33[b]	35[a]	36[a]	***	***	
Health related parameters						
Somatic cells (1000 L^{-1}))	172	225	194	NS	NS	NS
% cows receiving mastitis treatment	5.4[a]	2.5[b]	0.6[b]	**	NS	**
% cows receiving other health treatments	4.6	3.5	0.0	NS	NS	***
Feed composition parameters						
Mineral & vitamins supplements (g cow^{-1} day^{-1})	121[a]	3[b]	0[b]	***	NS	NS
Concentrate use (kg dry matter cow^{-1} day^{-1})	7.4[a]	0.8[b]	0.9[b]	***	0.07	*
Conserved forage intake as % DMI[¶]	45[a]	4[b]	0[b]	***	***	*
Grazing as % DMI[¶]	18[b]	92[a]	95[a]	***	***	***

Results with different superscript letters in the same row differ significantly.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[†] Main effects, [‡] interaction, [§] only recorded once per farm [¶] calculated values.
PS, production system; SD, sampling date; DMI, dry matter intake; NS, not significant.

previous studies[12,24–27] which show that other isomers (e.g. t12t14, t11t13, t9t11, t11c13, t8c10 and t7c9) are present in levels that could have a significant effect with respect to overall CLA levels in milk (Table 2).

For seven of the 14 CLA isomers assessed, significant differences were detected between production systems, with levels being lowest for the HI system, intermediate for the O-LI system and highest with the NO-LI system (Table 2), although differences between the two LI systems were only significant for total CLA and two isomers; the main c9t11, and t8c10.

Multivariate analysis showed that all seven of these isomers have a strong positive relationship with fresh forage intake (see Fig. 1). The remaining CLA isomers appear to be unaffected by production system and/or dairy diet. This group includes the five isomers present in relatively low concentrations (together forming only 0.11–0.16 mg g fat^{-1} representing 0.5–2.5% of total CLA concentration) as well as isomers t7c9 and t9c12 which are present at higher concentrations (0.35–0.39 and 0.17–0.26 mg g fat^{-1}, respectively) although the latter does show a trend ($P = 0.09$) of being influenced by the production system. The fact that the concentration of t7c9 is unaffected by production system and diet composition may be explained because, of all the isomers of CLA, it is unique in being produced solely by endogenous desaturation rather than by direct rumen synthesis.[20] However, as with isomer c9t11, secretion into milk is not independent of rumen biohydrogenation since synthesis is dependent on the supply of a rumen biohydrogenation intermediate (18 : 1 t7) as a precursor, in addition to desaturase activity in the mammary gland. The apparent difference between t7c9 and c9t11 in this respect raises the questions as to why concentrations of C18 t11 appear to be influenced by production system yet C18 : 1 t7 is not.

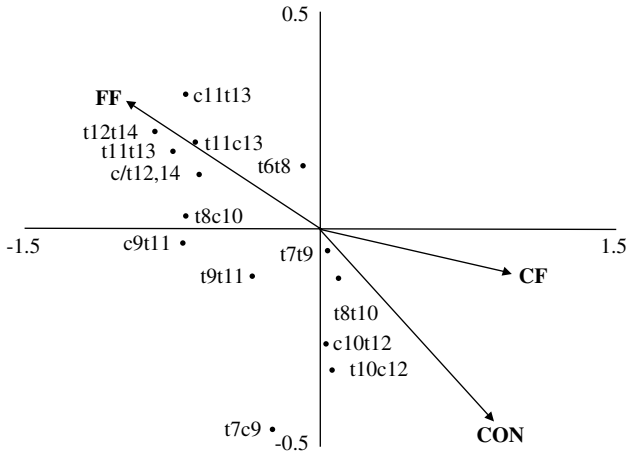

Figure 1. Biplots showing the distribution of individual CLA isomers in relation to the diet components of fresh forage (FF), conserved forage (CF) and concentrate (CON), derived from redundancy analyses.

For five (t12t14, t11t13, t12c14 + c12t14, t11c13 and c11t13) of the seven CLA isomers that were significantly affected by production system, the results are in accordance with those of Collomb *et al.*,[24] who found high correlation coefficients (0.74–0.89) between the concentration of these five CLA isomers and the daily intake of α-linolenic acid. Moreover, according to a hypothesis by Kraft *et al.*,[12] α-linolenic acid is an indirect precursor of the t11c13 isomer. Levels of this CLA isomer and/or the ratio of two isomers (t11c13/t7c9) were described as useful indicators of grass feeding, milk products of alpine origin[26,35] and/or other low-input systems. The increase in concentrations of these CLA

Table 2. Effect of (and interactions between) dairy production system [conventional high input (HI) and organically certified (O-LI) and non-organic (NO-LI) low input systems] and sampling date on the CLA isomers and associated fatty acid composition of milk fat (mg g fat^{-1})

		Production system			ANOVA results (*P* value)		
CLA isomer (mg g fat^{-1})		HI ($n = 16$)	O-LI ($n = 20$)	NO-LI ($n = 10$)	PS[†]	SD[†]	PSxSD[‡]
trans/trans CLA isomers							
1	t12 t14	0.12[b]	0.30[a]	0.35[a]	***	0.06	*
2	t11 t13	0.22[b]	0.68[a]	0.83[a]	***	**	0.06
3	t10 t12	0.05	0.04	0.03	NS	NS	NS
4	t9 t11[¶]	0.17	0.22	0.26	0.09	***	NS
5	t8 t10	0.03	0.03	0.01	NS	NS	NS
6	t7 t9	0.07	0.07	0.06	NS	***	**
7	t6 t8	0.01	0.02	0.01	NS	*	NS
cis/trans CLA isomers							
8	c/t 12,14	0.03[b]	0.07[a]	0.07[a]	***	*	0.05
9	t11 c13	0.22[b]	0.61[a]	0.80[a]	**	**	*
10	c11 t13	0.02[b]	0.04[a]	0.05[a]	**	NS	NS
11	t10 c12[¶]	0.02	0.02	0.01	NS	NS	NS
12	c9 t11[¶]	6.04[c]	10.72[b]	15.06[a]	***	***	**
13	t8 c10	0.12[c]	0.18[b]	0.22[a]	**	***	*
14	t7 c9	0.35	0.35	0.39	NS	*	NS
15	total CLA[§]	7.46[c]	13.33[b]	18.15[a]	***	***	**
Other fatty acids related to CLA metabolism							
Vaccenic acid	C18 : 1 t11	18.1[c]	34.7[b]	43.4[a]	***	*	*
Oleic acid	C18 : 1 c9	220	228	241	NS	NS	NS
Linoleic acid	C18 : 2 c9 c12	16.8	12.3	9.9	NS	NS	NS
γ-Linolenic acid	C18 : 3 c6 c9 c12	0.24	0.23	0.13	NS	NS	NS
α-Linolenic acid	C18 : 3 c9 c12 c15	5.4[b]	10.3[a]	9.4[a]	***	NS	NS
	C18 : 2 t11c15 + t9 t12	0.33	0.25	0.31	NS	NS	NS
$\Delta - 9$ desaturase activity[§]		0.28	0.28	0.29	NS	***	***

Linolenic acid; 18 : 2 t11 c15 was not determined.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[†] Main effects, [‡] interaction, [§] calculated value, [¶] isomers linked to biological activities.
PS, production system; SD, sampling date; NS, not significant.

isomers from HI to O-LI and to O-LI systems is also likely to be related to increased intakes of fresh forage (which is known to be rich in α-linolenic acid). Lower intake of concentrates for the O-LI and NO-LI systems than for the HI system (Table 1 and Fig. 1) are also known to increase the concentrations of the main CLA isomers.[28]

On the other hand, the concentration of the main CLA isomer c9t11, in milk has been previously shown to increase with increasing intakes of linoleic rather than α-linolenic acid.[24] It is well known that the pathway for biohydrogenation of α-linolenic acid (C18 : 3 c9c12c15) in the rumen involves an initial isomerisation to a conjugated triene (C18 : 3 c9t11c15), followed by a reduction of double bonds at carbons 9, 15 and 11 to yield the fatty acids C18 : 2 t11c15, C18 : 1 t11 and C18 : 0, respectively.[36] Dietary linoleic acid (18 : 2 *cis*-9,*cis*-12) is first isomerised to the CLA *cis*-9,*trans*-11 by *cis*-12,*trans*-11 isomerase and then hydrogenated by *Butyrivibrio fibrisolvens* to vaccenic acid (VA, *trans*-11 18 : 1) in the rumen.[37] This main *trans*-FA is responsible for the formation of the CLA isomer c9t11, which occurs by desaturation (Δ^9-desaturase) of the ruminally derived VA in the mammary gland.[19]

Interestingly, the only other isomer of CLA to differ significantly between the two LI systems was t8c10, which has also been shown to correlate with intakes linoleic rather than α-linolenic acid.[24]

As well as significant influences of production system on concentrations of seven of the CLA isomers, there was also a trend ($P = 0.09$) for higher levels of isomer C18 : 2 t9t11 in milk from the LI systems. This isomer has been linked to lower cancer incidence,[18] and has been shown to be influenced by intakes of LA, although to a lesser extent than c9t11 with coefficients of correlation at 0.58 and 0.81, respectively.[24]

Although chemical composition of the dairy diets were not assessed in this study, the CLA profiles associated with different production systems suggest that cows under both LI systems had higher intakes of linoleic and α-linolenic acid than those under HI management. In addition, the gradient in concentrations of c9t11 and t8c10 in milk suggests cows on the NO-LI system had higher intakes of linoleic acid in comparison to those under O-LI management. It would therefore be important to assess fatty acid concentrations of the main dietary ingredient in future studies.

There was no significant difference in the levels of CLA isomer C18 : 2 t10c12 between production systems, with levels found to be very low (<0.03 mg g^{-1} fat) on all sampling dates. Low levels of this isomer C18 : 2 t10 c12 in milk are desirable, because (1) increasing levels of this isomer in the udder (e.g. through post-ruminal infusion of synthetic CLA, which includes high levels of

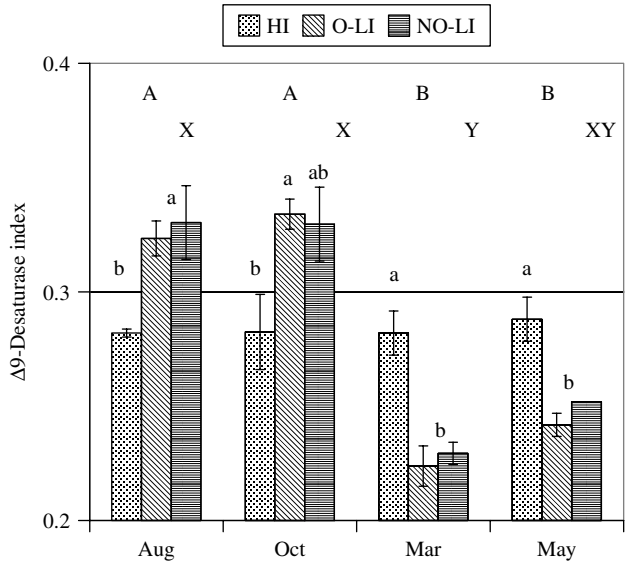

Figure 2. Δ9-desaturase index ([CLA c9, t11]/([VA] + [CLA c9t11]) at different times of the year in milk from organically certified low input (O-LI), non-organic low input (NO-LI) and conventional high input (HI), herds. For each sampling date means for different production systems were compared; those with the same lower case letter do not differ significantly ($P < 0.05$). For each production system, means for each date were compared and those with the same upper case letter do not differ significantly ($P < 0.05$, with A and B for O-LI, and X and Y for NO-LI).

t10t12) was associated with significant reductions in yield and lactose concentration, and elevated cell counts in milk;[13] and (2) high dietary intake of isomer C18 : 2 t10 c12 has been linked to negative as well as positive health impacts.[2]

These data along with previous studies of the effect of forage and oil-seed supplements on CLA isomer composition in milk, reviewed by Collomb *et al.*[3] and confirmed in subsequent studies,[25,27] provide the tools to produce milk with contrasting isomer composition for future dietary intervention studies.

Effect of sampling date on CLA isomers in milk fat

For total CLA and nine of the 14 CLA isomers assessed significant differences were also detected between sampling dates (Tables 2 and 3). These differences were very highly significant ($P < 0.001$) for two of the isomers (c9t11 and t9t11) (Tables 2 and 3), which were linked to potential beneficial effects on human health. Total CLA levels in milk from the two LI systems (O-LI and NO-LI) were lowest in March, slightly increased in May, at the highest level in August and then slightly lower again in October (Table 3). According to Stanton *et al.*[38] higher intakes of fresh grass increased the total CLA content of milk, a finding also reported by Kelly *et al.*[39] and Dhiman *et al.*[40] who also found that the CLA content of milk increased in cows when moving from preserved to fresh grass diets. Most individual CLA isomers for which significant differences between sampling dates were detected (including c9t11, which accounted for more than 80% of CLA) followed the same pattern over time as that of total CLA concentration (Table 3).

In contrast to the widely fluctuating CLA content of LI milk, levels of total CLA in milk from the HI farms were generally lower and more consistent (ranging between 6 and 9 mg g^{-1} fat) between sampling dates (Table 3). The lower CLA levels from HI farms was thought to be due to the lower levels of fresh grass combined with higher levels of silage and concentrates in the feeding regimes used on these farms, since CLA levels were previously shown to increase with increasing levels of fresh grass and to decrease with increasing levels of concentrates and grass silage in the dairy diet.[8,22,28]

Significant interactions between production system and sampling date were detected for five of the 14 CLA isomers included in the study; being highly significant ($P < 0.001$) for total CLA and the most abundant isomer c9t11 (Table 2). While levels of total CLA, and isomer c9t11, in milk were similar for both LI systems in October, March and May, for August they were significantly higher in milk from the NO-LI system.

Table 3. Effect of (and interactions between) dairy production system [conventional high input (HI) and organically certified (O-LI) and non-organic (NO-LI) low input systems) and sampling date on the CLA isomers composition of milk fat (mg g fat^{-1}; only CLA isomers for which sampling date had a significant effect are shown)

CLA isomer		Sampling date											
		August 2004			October 2004			March 2005			May 2005		
mg g fat^{-1}		HI ($n = 4$)	O-LI ($n = 5$)	NO-LI ($n = 3$)	HI ($n = 4$)	O-LI ($n = 5$)	NO-LI ($n = 3$)	HI ($n = 4$)	O-LI ($n = 5$)	NO-LI ($n = 3$)	HI ($n = 4$)	O-LI ($n = 5$)	NO-LI ($n = 1$)
1	t12 t14	0.14^{bcd}	0.32^{a}	0.43^{a}	0.10^{d}	0.26^{abcd}	0.38^{a}	0.11^{d}	0.33^{a}	0.26^{abcd}	0.14^{cd}	0.29^{abc}	0.33^{abcd}
2	t11 t13	0.21^{de}	0.80^{ab}	0.89^{ab}	0.17^{e}	0.63^{bcd}	0.80^{ab}	0.20^{e}	0.57^{bcde}	0.57^{bcde}	0.31^{cde}	0.71^{bc}	1.47^{a}
4	t9 t11	0.23^{ab}	0.32^{ab}	0.40^{a}	0.12^{b}	0.25^{ab}	0.25^{ab}	0.15^{b}	0.13^{b}	0.12^{b}	0.16^{b}	0.18^{ab}	0.29^{ab}
6	t7 t9	0.07^{abc}	0.09^{ab}	0.08^{abc}	0.06^{abc}	0.09^{a}	0.08^{abc}	0.07^{abc}	0.04^{c}	0.03^{c}	0.07^{abc}	0.05^{bc}	0.05^{abc}
8	c/t 12,14	0.03^{c}	0.10^{a}	0.08^{ab}	0.02^{c}	0.07^{ab}	0.08^{ab}	0.03^{bc}	0.06^{abc}	0.05^{bc}	0.03^{bc}	0.06^{abc}	0.08^{abc}
9	t11 c13	0.31^{cde}	0.78^{abcd}	1.07^{a}	0.17^{e}	0.60^{abcde}	0.95^{ab}	0.16^{e}	0.59^{abcde}	0.51^{abcde}	0.25^{de}	0.48^{bcde}	0.44^{abcde}
10	c11 t13	0.02^{c}	0.04^{a}	0.05^{a}	0.02^{c}	0.03^{abc}	0.04^{ab}	0.02^{c}	0.04^{ab}	0.04^{ab}	0.02^{bc}	0.04^{abc}	0.06^{a}
12	c9 t11	7.0^{cde}	12.9^{bc}	21.2^{a}	5.3^{de}	10.9^{bcd}	15.7^{ab}	4.6^{e}	9.2^{cde}	9.9^{cde}	7.3^{cde}	9.9^{cde}	10.4^{abcde}
13	t8 c10	0.13^{bc}	0.21^{ab}	0.30^{a}	0.10^{c}	0.18^{bc}	0.22^{ab}	0.09^{c}	0.15^{bc}	0.16^{bc}	0.15^{bcd}	0.17^{bc}	0.20^{abc}
14	t7c9	0.37	0.33	0.45	0.34	0.36	0.40	0.31	0.30	0.33	0.39	0.39	0.30
14	Total	8.7^{cde}	15.9^{bc}	25.0^{a}	6.4^{de}	13.6^{bcd}	18.9^{ab}	5.8^{e}	11.5^{cde}	12.0^{bcde}	8.9^{cde}	12.4^{bcd}	13.7^{abcde}

For each production system and sampling date, means were compared by Tukey's honest significant difference test, and those with the same letter do not differ significantly ($P < 0.05$).

Divergence between the two LI systems is likely to be due to differences in the sward composition, which was previously reported to affect both total CLA levels and isomer profiles.[24] Since farms from both LI systems were in the same geographic region (south-west Wales) and used grass–clover swards for grazing, the main differences between swards are thought to have been either in herbage availability or the proportion of clover in the swards as a consequence of applications of mineral nitrogen fertilisers (up to 120 kg ha^{-1}) to swards on NO-LI farms. This practice is known to reduce the relative competitiveness and proportion of clover in grass–clover leys.[41]

Conversion of vaccenic acid to CLA c9, t11

With respect to endogenous synthesis of CLA, the Δ9-desaturase index has been described as the ratio between product and total potential substrate;[42] in this case, the CLA isomer c9t11 relative to vaccenic acid plus c9t11 isomer. Overall, the Δ9-desaturase activity does not differ between production systems (Table 2); however, there is a highly significant effect of sampling date and interactions between system and date, which is illustrated in Fig. 2. Compared to the HI system (which calved all year round), where the Δ9-desaturase activity was similar on all sampling dates, the index calculated for the two LI systems is significantly higher in milk from August and October samples compared with that taken in the spring. This could be a 'stage of lactation' effect on conversion of vaccenic acid to CLA c9,t11 since both LI herds are block calved in February and March (low values) and all cows were in late lactation when the high values were recorded in autumn. However, since diets on both LI systems were dominated by grazing, and sward quality was not assessed in this study, a dietary influence (related to differences in sward composition) on desaturase activity or availability of the VA substrate can not be excluded. This needs to be investigated in future studies.

Effect of components of the production systems on milk quality parameters (multivatiate analyses)

Initial RDA analyses based on indices for diet, health and breed parameters showed that only diet-related components (diet index) influenced the levels of CLA isomers in milk fat (analyses not shown).

This confirms previous experimental studies and reviews, which concluded that CLA levels are closely correlated to feeding regimes.[28,43,44] However, it should be noted that the breed index was closely correlated with both feeding regime (with the two LI farms using cross-bred and the HI farms using pure Holstein Friesian animals). Future studies should therefore examine in more detail the possible effects of breeds, crosses and/or breeding strategies on CLA levels in milk.

The eigenvalues and the cumulative relationship values (%) derived from RDA analyses between the dietary components (fresh forage, conserved forage and concentrates) and CLA isomers are shown in Table 4. The eigenvalues and cumulative relationship values show the paramount influence of axis 1 in all analyses, with very little variation explained by axis 2.

The RDA biplot (Fig. 1) shows the relationship between the three individual diet components (fresh forage, conserved forage and concentrates) and individual CLA isomers. All three dietary components were more strongly related to axis 1 than to axis 2, although both fresh forage and concentrates showed some allegiance to axis 2. Conserved forage and concentrates were both situated along the positive axis 1, opposite to fresh forage. The 14 CLA isomers assessed fell into 3 groups with respect to this comparison. The majority of CLA isomers which were influenced by production system (t12t14, t11t13, c/t1214, t11c13, c11t13, t8 c10) were clustered along the fresh forage line and associated with the negative axis 1 and therefore allied with a high proportion of fresh forage in the diet. This could indicate that the relative efficiency of the rumen microbial flora in generating these CLA isomers and/or the supply of their precursors is increased when fresh forage based diets are used.

Table 4. Eigen values and the cumulative relationships (%) between CLA isomers and diet components (fresh forage, conserved forage, concentrates) for the first two axes of the RDA analyses

	Axis 1	Axis 2
Eigenvalue	0.4244	0.0004
Cumulative CLA isomer/diet component relationship	99.9	100.0

In contrast, most CLA isomers that were not significantly affected by production system (t10t12, t8t10, t7t9, t6t8, t12c12), were very close to axis 2 (Fig. 1), indicating a lack of strong association with any of the feed components. However, isomers t8 t10, c10 t12 and t10 c12 from this group were closest to the concentrate line, indicating a weak association with concentrate level in the diet. Previous studies have shown excessive concentrate and/or low forage diets result in an increase in CLA isomer t10c12. This isomer has been linked to both positive and negative health impacts in humans and was shown to reduce milk fat synthesis and body fat gain in dairy cows when applied via post-ruminal infusion of synthetic CLA.[13] HI herds included in this study were feed an average of 7.4 kg concentrate dry matter per cow per day and perhaps this relationship would have been stronger if herds feeding even higher levels of concentrates had been included.

The third group of isomers (c9t11, t7c9 and t9t11) showed different associations. The major (>80% of total) isomer of CLA found in milk, c9t11, is not as closely associated with the fresh forage line in Fig. 1 as other isomers that were significantly influenced by system (see Tables 2 and 3) yet does have a strong negative relationship with axis 1. This may be explained by differences in its biosynthesis (compared to other isomers), predominantly produced endogenously from vaccenic acid.[19] The only other isomer created by endogenous synthesis, t7c9, (which was not affected by production system) had the greatest negative value on axis 2, but relatively close to axis 1. The other exception was t9t11, which showed a trend for being affected by production system ($P = 0.09$), yet does not appear to be influenced strongly by any of the three dietary components. This isomer, along with c9t11, has been linked to beneficial health impacts.[2,18]

Potential health impacts of consumption of milk with increased CLA levels

Whilst the results reported here, together with evidence from other studies,[12,24–27] clearly indicate that production systems and/or the season of production influence the CLA profile in milk fat, there is still a lack of animal or human dietary intervention studies into the potential effects of consuming milk from organic and other LI systems on animal or human health. There is

evidence that consumption of organic dairy produce elevates total CLA level in breast milk[9] and for a reduced risk of childhood eczema associated with consumption of organic dairy produce;[10] however, additional studies are necessary to link such benefits to detailed compositional differences detected in studies such as this. Ruminant fat is known to be the primary source for the intake of CLA and given the decrease in consumption of red meat and the demand for leaner beef and lamb products, the intake of CLA from dairy products could be of increasing importance. The effect of consuming milk and dairy products with different levels of CLA should therefore be investigated in detail in future studies.

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