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Evidence That Forage-Fed Cows Can Enhance Milk Quality

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Abstract: Researching the distinguishing factors of nutritional milk quality is key to sustainable production and addresses increasing media and scientific scrutiny regarding human health effects and ecological impacts of dairy products. Modern Western diets have high omega-6 relative to omega-3 fatty acid (FA) consumption. This ratio in milk can be manipulated by management practices; increasing forage in dairy diets raises omega-3 in milk. Whilst studies identify higher concentrations of nutritionally beneficial FAs in organic dairy, milk from 100% forage-fed cows in the UK has not been investigated. This study explores differences in FA composition between supermarket conventional and organic and Pasture for Life Association (PFLA) milk, collected in April, July and October, 2017. PFLA milk had higher concentrations of conjugated linoleic acid (+94%) and omega-3 (+92%) than conventional milk. Additionally, concentrations of palmitic acid (+11%), omega-6 (+69%) and the ratio of omega-6/omega-3 (+201%) were higher in conventional than PFLA milk. PFLA milk had higher concentrations of alpha-linolenic acid (+39%), conjugated linoleic acid (+30%) and omega-3 (+21%) and lower concentrations of omega-6 (−36%) and a lower ratio of omega-6/omega-3 (−44%) than organic milk. This supports previous studies and demonstrates the scope to improve milk FA profiles further for potential health benefits through pasture-based management.

Keywords: dairy; fatty acids; organic; pasture; forage; milk quality

1. Introduction

Fats from dairy products have undergone major scrutiny by both the media and scientific community. Though ruminant agriculture has been criticised for its land use and emissions, a recent study indicates that lower-yielding, pasture-based production can have lower emissions per litre of milk compared to more-intensive, higher-yielding systems [1]. Additionally, despite claims that dairy products are unnecessary sources of saturated fat and calories, dairy fats are a complex of important fatty acids (FAs) [2], many beneficial to human health. The more forage that cows consume, the higher the concentration of beneficial fatty acids [3,4], linking environmental benefits with nutritional gains.

Long-chain omega-3 FAs (n-3)—eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)—have been shown to lower the risk of coronary heart disease (CHD) and are anti-inflammatory, anti-thrombotic and anti-atherogenic [5,6]. Additionally, long-chain FAs are essential in brain development and function and have protective effects that potentially support healthy aging [7,8]. Conjugated linoleic acid (C18:2, c9t11 isomer—CLA9) was also linked to a lower CHD risk and enhances immune response, improves body composition and reduces certain cancers and infant eczema [9,10]. The most abundant n-3 in milk, alpha-linolenic acid (C18:3 c9,12,15—ALA), which converts to EPA, DPA and DHA, has been associated with healthy aging and foetal development [6,11]. However, there are also FAs present in dairy fat with a negative effect on human health. Lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) increase CHD risk [12,13]. Further, whilst linoleic acid (LA), the most abundant polyunsaturated fatty acid (PUFA) and omega-6 (n-6), is important for healthy skin [14], in excess, dietary n-6 have been linked to an increased risk of obesity and pro-inflammatory effects [15,16]. Finally, an elevated ratio of n-6/n-3 comes with a higher risk of type 2 diabetes, increased CHD and obesity [16–18].

Dairy herd management has the biggest impact on milk fat composition; cows fed a forage-based diet produce milk with more nutritionally beneficial FAs compared to grain-fed herds [4,19–22]. The Pasture for Life Association [23] is a UK certifying body that ensures 100% grass and forage-fed (maize and maize silage is not permitted) high-welfare beef, lamb and dairy products. PFLA farmers choose robust breeds that stay healthy and reach maturity under pasture-based forage-only diets and utilise diverse vegetation swards consisting of herbs, red clover, grasses and wild flowers. This is designed to reduce costs, increase profitability and regenerate and improve soil fertility, farm land and the environment [23]. A recent nationwide American study [3] compared conventional, organic and Grassmilk® (a trademark label from Organic Valley (Wisconsin, USA), similar to PFLA certification) milk quality and found significantly different FA profiles in the three milk types. Grassmilk® was higher in n-3, CLA9 and EPA and had a lower n-6/n-3 ratio, attributes shown to lower cardiovascular and metabolic diseases [6,24]. These recent findings suggest a marked difference in the nutritional quality of pasture-only milk, with the potential to consume more FAs beneficial for human health, which has yet to be investigated in the UK.

This paper aims to explore differences in milk fat composition between own-brand conventional and organic milk from supermarkets and PFLA milk in the UK.

2. Methods

2.1. Experimental Design

Milk was collected from eight PFLA dairy farms (across southern UK) and five supermarkets (located centrally to the PFLA farms—within 10 miles (16 km) West of Bristol) in April (4^{th}–6^{th}), July (4^{th}–6^{th}) and October (10^{th}–12^{th}), 2017. There is no winter sampling because PFLA farmers predominantly spring calve, and therefore cows do not produce milk from November to January/February. The locations of the PFLA farms and the supermarkets are in Appendix A Figure A1. A representative 500 mL milk sample was taken from the bulk tank on each farm (after stirring to disperse the cream) on the morning of collection. This coincided with the purchase of half-litre (500 mL), own-brand organic and conventional whole, pasteurised and homogenised milk from five supermarkets: Asda, Marks and Spencer, Sainsbury's, Tesco and Waitrose. None of the dairy farms supplied milk to the supermarkets.

2.2. Milk Analysis

All samples were kept below 4 °C during transportation (maximum 3 days) and then frozen at −20 °C until analysis. Milk from supermarkets was purchased on the same day as each farm sampling date. All were in date and their shelf life went beyond the date they were frozen. The samples were thawed overnight at 4 °C, freeze dried and then 130 µg of lyophilized milk was methylated and esterified to prepare for gas chromatography (GC), using the method described by Chilliard et al. [25] and Stergiadis et al. [26]. The chemicals used for extraction of FAs, correction factors for short-chain FAs (C4:C10:0), analytical standards and identification of peaks followed the methodology of and are described by Stergiadis et al. [27].

Fatty acid methyl esters (FAMEs) were separated and quantified using GC (Shimadzu, GC-2014, Kyoto, Japan). The GC had a flame ionisation detector and a 100 m × 0.25 mm ID, 0.2 µm film thickness, Varian CP-SIL 88 fused silica capillary column. To optimize peak separation, modifications to the chromatographic conditions from the original method by Chilliard et al. [25] were updated as described by Stergiadis et al. [27].

Although purchased milk was pasteurised and homogenised and farm milk was in a raw state at sampling, milk FA profiles are not changed by these processes [28], making this an appropriate comparison. There is some evidence that time after pasteurisation could reduce CLA concentration [29]. However, further research is required to confirm this. Fatty acid results are expressed as a percentage of the whole FA profile. Thus, there is no need to adjust for differences in fat content between the whole supermarket milk (3.5% fat) and the raw farm milk (~3.5%–4.5% fat).

2.3. Data Analysis

Microsoft Excel was used for data handling and statistical analysis was completed using the statistical software 'R' [30]. Tests included a linear mixed-effects model (R package 'nlme') to allow for nested random effects and unbalanced design, analysis of variance and Tukey's honestly significant difference. The factors were month (April, July, October (n = 18 each month)) and management (conventional (n = 15), organic (n = 15), PFLA (n = 24)), with source (supermarket (own-brand) name and farm name) treated as a random factor. The concentrations of FAs identified as having either a positive or negative impact on human health are explored in the results.

3. Results

Milk fat composition varied by management system but rarely by month and interactions between management and month were not significant. The concentrations of the main nutritionally relevant FAs are presented in Table 1, although full details of the FA profiles are presented in Appendix A, Table A1. Unless stated otherwise, all differences mentioned here and in the discussion were significant ($p < 0.05$).

Table 1. Effect of management (conventional, organic and PFLA) and month (April, July and October) on the concentrations of nutritionally relevant fatty acids (FAs) in milk. Mean FA proportion, standard deviation (SD) and ANOVA *p*-values for each FA, expressed as a percentage of the entire FA profile.

	Management			**Month**			***p*-Value**		
	Conv.	**Org.**	**PFLA**	**April**	**July**	**October**	**Man**	**Mon**	**Man × Mon** †
Fatty Acids	n = 15	n = 15	n = 24	n = 18	n = 18	n = 18			
ALA ‡	$0.47^{c} \pm 0.020$	$0.79^{b} \pm 0.032$	$1.1^{a} \pm 0.06$	0.79 ± 0.066	0.82 ± 0.078	0.86 ± 0.083	***	ns	ns
CLA9	$0.62^{c} \pm 0.018$	$0.92^{b} \pm 0.036$	$1.2^{a} \pm 0.09$	0.95 ± 0.118	0.90 ± 0.056	0.98 ± 0.082	***	ns	ns
n-3	$1.2^{c} \pm 0.05$	$1.9^{b} \pm 0.10$	$2.3^{a} \pm 0.10$	1.8 ± 0.13	1.8 ± 0.14	2.00 ± 0.14	***	ns	ns
EPA + DHA + DPA	$0.25^{b} \pm 0.022$	$0.39^{a} \pm 0.038$	$0.38^{a} \pm 0.016$	0.36 ± 0.032	0.34 ± 0.028	0.35 ± 0.025	**	ns	ns
C12:0	3.5 ± 0.06	3.3 ± 0.04	3.4 ± 0.12	$3.6^{a} \pm 0.11$	$3.3^{b} \pm 0.08$	$3.3^{b} \pm 0.08$	ns	**	ns
C14:0	11.0 ± 0.10	11.0 ± 0.08	11.1 ± 0.24	11.2 ± 0.22	11.0 ± 0.21	11.0 ± 0.14	ns	ns	ns
C16:0	$31.1^{a} \pm 0.30$	$28.6^{b} \pm 0.32$	$28.0^{b} \pm 0.80$	29.0 ± 0.94	28.6 ± 0.64	29.5 ± 0.54	*	ns	ns
n-6	$2.7^{a} \pm 0.06$	$2.5^{a} \pm 0.06$	$1.6^{b} \pm 0.09$	2.1 ± 0.15	2.1 ± 0.13	2.2 ± 0.14	***	ns	ns
n-6/n-3	$2.2^{a} \pm 0.10$	$1.3^{b} \pm 0.06$	$0.73^{c} \pm 0.021$	1.4 ± 0.17	1.3 ± 0.16	1.2 ± 0.14	***	t	ns

Notes: † a–c Mean values for management or date in a row with different letters are significantly different, according to Tukey's honestly significant difference test (*p*-values < 0.05). ***: $p < 0.001$, **: $p < 0.01$; *: $p < 0.05$, t: $p < 0.1$, and ns: $p > 0.1$. ‡ ALA = alpha-linolenic acid, CLA9 = conjugated linoleic acid (C18:2, c9t11 isomer), n-3 = omega-3, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid and DHA = docosahexaenoic acid, C12:0 = lauric acid, C14:0 = myristic acid, C16:0 = palmitic acid and n-6 = omega-6. Conv. = conventional, Org. = organic, PFLA = Pasture for Life Association, Man = management, Mon = month, and SD = standard deviation.

3.1. Effect of Management on Composition

Generally, nutritionally beneficial FAs had a gradient in concentrations, being lowest in conventional supermarket milk and highest in the PFLA milk (Table 1), with organic supermarket milk intermediate. There were higher concentrations of ALA (+134%), CLA9 (+94%), n-3 (+92%) and EPA + DPA + DHA (+52%) in PFLA milk compared with conventional milk. There were also higher concentrations of C16:0 (+11%) and n-6 (+69%) and a higher ratio of n-6/n-3 (201%) in conventional milk compared to PFLA milk. When compared to organic milk, PFLA milk had higher concentrations of ALA (+39%), CLA9 (+30%) and n-3 (+21%), lower concentrations of n-6 (−36%) and a lower ratio of n-6/n-3 (−44%). Additionally, organic milk had higher concentrations of ALA (+41%), CLA9 (+33%), n-3 (+37%) and EPA + DPA + DHA (+36%) but lower concentrations of C16:0 (−8%) and a lower ratio of n-6/n-3 (−41%) compared to conventional milk.

3.2. Effect of Sampling Month on Composition

The only FA that showed seasonal variation was C12:0, which was ~8% higher in April compared with milk collected in July and October.

3.3. Effect of Management and Month

There was no significant interaction between management and sampling month for the fatty acid profiles. However, Figure 1 demonstrates that for conventional and organic milk, the ratio of n-6/n-3 was significantly higher in April compared with other months. PFLA milk had a more consistent ratio across the three sampling dates and also had greater consistency between the individual farms compared with variation between samples bought from the various supermarkets (as indicated by the standard deviation).

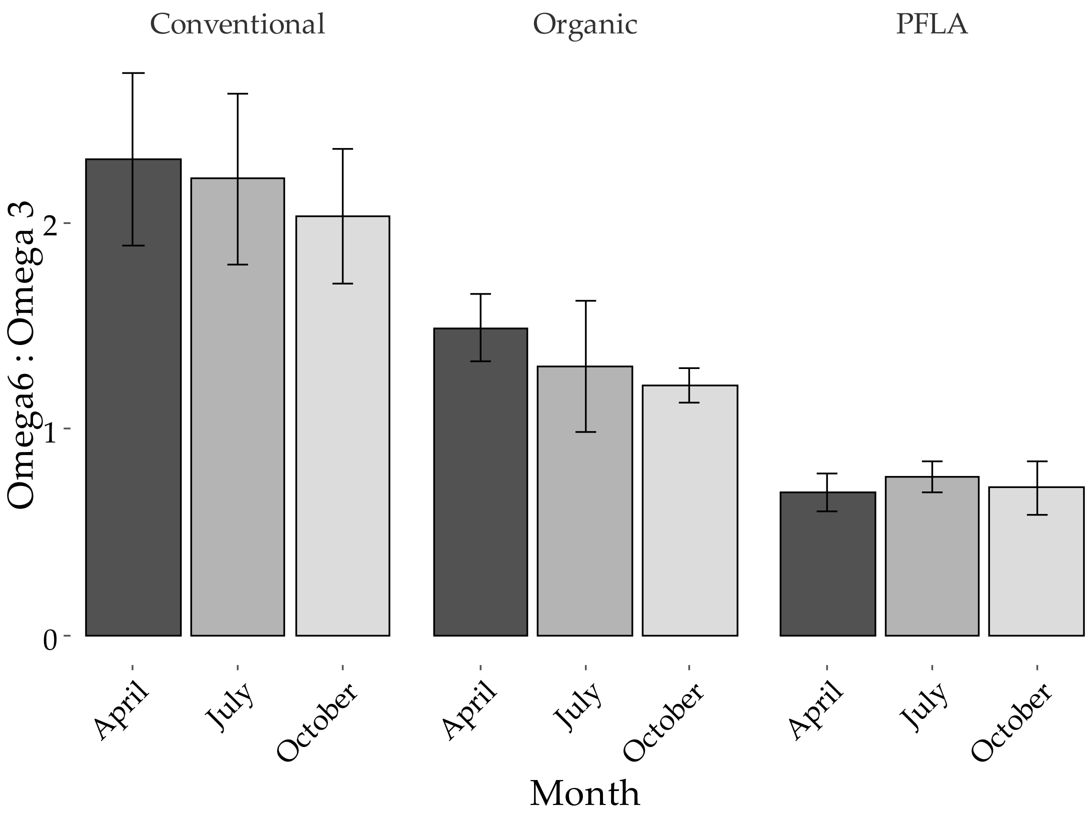

Figure 1. Ratio of n-6/n-3 by month and management from conventional and organic supermarket and PFLA milk (mean values ± SD).

4. Discussion

4.1. Milk Fat Composition

This study explored differences in milk fat composition between conventional and organic UK supermarket own-brand and PFLA products and found significant differences between the three types of milk. Overall, these findings support previous studies (summarised in Table 2), showing benefits in organic compared with conventional milk but also, for the first time, demonstrate the scope for further improvement beyond that of typical organic milk in the UK through forage-only feeding.

4.1.1. Omega-6 and Omega-3

The main n-6 (LA) and n-3 (ALA) PUFAs must both come from our diet, as they cannot be synthesised by humans. However, the balance of these two essential FAs is important. Both are metabolised into long-chain FAs—LA to arachidonic acid and ALA to EPA, DPA and DHA. However, ALA and LA compete for the same enzymes for elongation/desaturation, resulting in lower EPA, DPA and DHA generation from ALA at high dietary LA intake levels [16]. In the past 150 years, the n-6/n-3 ratio in our diet has increased dramatically from approximately 1/1 to 10–25/1. Historically, n-3 came from meat (reared on pasture), fish, leafy green vegetables, nuts and berries. However, this has decreased, whilst n-6 consumption has increased with the inclusion of vegetable oils (including sunflower, soya bean, and corn/maize oil), cereal grains and animal products produced from grain-based, rather than forage-based diets over the last 50 years [31]. There is increasing evidence that this excess n-6 consumption and increase in dietary n-6/n-3 has contributed to a sharp rise in obesity, atherosclerosis and diabetes [15,16,32]. Therefore, working towards a n-6/n-3 ratio closer to 1–4/1 is thought to be important for human health [31]. While, even the n-6/n-3 ratio of conventional milk (2.2) was within the recommended range, it was three times lower in PFLA grass-fed milk (0.73)—more n-3 than n-6, with the potential to counterbalance high n-6 intake levels in many Western diets.

Results from other studies presented in Table 2 show a lower n-6/n-3 ratio in organic milk compared with conventional milk, and an even lower ratio for milk from low-input/forage-based systems. Details of the diet and management system used on farms producing the conventional and organic supermarket milk assessed in this study were not available. However, results from previous farm surveys including feeding details (Table 2) also showed clear positive associations between the ratio of n-6/n-3 and forage consumption in dairy diets. For example, PFLA-type farms producing Grassmilk® in the US [3] showed that the highest proportion of forage in the diet had the lowest n-6/n-3 ratio (0.95) and low-input, organic farms that used moderate levels of concentrate had an intermediate ratio (2.3), while milk from conventional feedlot dairy systems had the highest n-6/n-3 ratio (5.8). It is well known that the inclusion of grain in livestock diets increases n-6 and suppresses n-3 in both dairy and meat produce [3,21,33]. Additionally, modelling by Benbrook et al. [3] suggests that a dietary switch from moderate consumption of conventional dairy products to high consumption of Grassmilk® products reduces the LA/ALA ratio (LA and ALA are the major PUFAs, and this ratio tends to mirror that of n-6/n-3) in the total diet by 47%, hypothesised to have a direct benefit to human health. The milk collected from PFLA farms here (n-6/n-3: 0.73) show similar results to those reported for Grassmilk® in the US (n-6/n-3: 0.954) by Benbrook et al. [3] and other studies (Table 2), suggesting that these systems maximising grazing and other forages in dairy diets significantly alter the FA composition of milk, providing potential health benefits to consumers.

Table 2. The omega-6/omega-3 ratio from studies examining milk fat composition across management systems both at the farm and retail level.

Source	Location	Management	Omega-6/Omega-3	Reference
Retail studies	NE England	Conventional Organic	3.8 2.6	[28]
	USA	Conventional Organic Grassmilk®	5.8 2.3 0.95	[3]
	England	Conventional Organic	2.2 1.3	Current study
	South England	Conventional Organic	2.6 1.7	[34]
Farm surveys	NW England and Wales	Conventional Organic	2.5 1.5	[20]
	UK	Conventional Organic Conventional Low-Input †	2.7 1.3 1.1	[19]
	Denmark UK	Conventional Organic Low-Input	4.7 1.9 1.0	[35]
	Wales	Conventional Organic Conventional Low-Input	3.1 1.7 1.4	[4]
	USA England	Grassmilk PFLA	0.95 0.73	[3] Current study

† Conventional low-input farms are not certified organic but generally follow some organic principles, often are spring calving and feed very little or no concentrate during lactation (the outdoor season), but, unlike organic farms, may use nitrogen fertiliser.

4.1.2. Conjugated Linoleic Acid

The main CLA in cow's milk is the isomer CLA9, which is almost exclusively from ruminant animal fats including dairy products [36]. This study found higher concentrations of CLA9 in milk from cows on pasture, and this is supported by previous research [37,38]. Milk from conserved forage diets has lower concentrations of CLA than milk from cows grazing fresh forage, although many studies have supplemented silage-based dairy diets with oilseeds, fish and/or various vegetable oils to improve the milk FA profile by elevating CLA concentrations [25,36,39]. In this study, the 100% grass-fed milk (1.2%) had double the concentration of CLA9 compared to the conventional supermarket milk (0.62%), suggesting that it may not be necessary to feed expensive oils or seeds, when high-quality, fresh forage could be used.

4.2. Effect of Management and Season

Seasonal variation in milk composition is expected due to the difference in feeding throughout the year on most systems (often cows are outdoors grazing during the summer and indoors on silage or hay diets during winter) [19]. Different patterns in the ratio of n-6/n-3—conventional and organic milk follows the expected trend of a high ratio of n-6/n-3 in April (when high-yielding cows might still be fed conserved forages with grains or cereal by-products) and a decrease in the ratio across July and October, when diets are likely to have increasing proportions of fresh forage from grazing (Figure 1). In both the conventional and organic milk, n-3 concentrations gradually increased across the three sampling dates, whilst n-6 either remained the same (conventional) or decreased (organic), which is a similar pattern reported by Butler et al. [28] and Kliem et al. [40] (only conventional) in seasonal retail milk comparison studies. However, the PFLA milk did not follow this pattern; both n-3 (higher in PFLA than the other milks) and n-6 (lower than the other milks) increase incrementally from

July to October, resulting in the n-6/n-3 ratio remaining relatively constant. PFLA farms were spread across the southern UK, with a wide range of herd sizes, breeds, sward types and management, but the main constant between farmers was their feeding policy. PFLA does not feed any grains, only forage, suggesting the continuity in forage-based feeding creates a more consistent composition, especially in the ratio of n-6/n-3, which has a stronger effect than location or other aspects of management (e.g., breed). Although the supermarket milk samples may have come from a smaller geographic area, they had a wide variation, partially attributed to the unknown of how many farms contributed to each sample. Despite this inherent variability, likelihood is high (especially in conventional systems) that cows come from a similar genetic lineage, suggesting that management, including feeding, is diverse in farms supplying the different supermarkets.

4.3. Forage in the Diet

A common theme of this paper is that concentrations of nutritionally beneficial FAs were significantly higher with pasture-based feeding. Fatty acid manipulation/improvement has been extensively researched with oils [39] and/or conserved forage [41]. However, evidence from this paper and work by Benbrook et al. [3] and others (Table 2) demonstrate that fatty acid profiles can improve by increasing pasture within dairy diets. This could be a cheaper and more effective way of modifying FA profiles whilst supporting the natural function of cow digestion and increasing beneficial FAs in the human diet. Increasing forage (and decreasing concentrate feed) could lower yields but the cost saved on inputs could be worth the compromise. Thus, a full economic analysis is required. The PFLA farm business case for feeding ruminants solely on pasture and conserved forages [42] finds that, with the right management, raising ruminants on forage can be profitable. Additionally, in the USA, Grassmilk® farmers receive a 15% premium (over and above that for organic milk) if concentrations of beneficial FAs reach a threshold [3]. Whilst this is not yet available in the UK, interest in the nutritional composition of foods and encouraging high welfare and sustainable farming standards is high.

5. Conclusions

In conclusion, this study has found that milk from 100% forage-based dairy systems has a nutritionally more desirable FA profile compared to conventional and organic supermarket milk. Of particular interest is the ratio of n-6/n-3, which decreases dramatically from conventional to organic to PFLA milk. Additionally, the ratio of n-6/n-3 remained constantly low over the seasons in PFLA milk yet peaked (end of winter) and troughed (end of summer) in conventional and organic milk. Whilst these findings are consistent with previous studies, the novel finding in this study shows further improvement in the FA profile beyond organic milk. This provides scope for further and larger-scale research into producing 'more nutritious' milk and how dairy consumption can alter the ratio of n-6/n-3 in the total diet.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

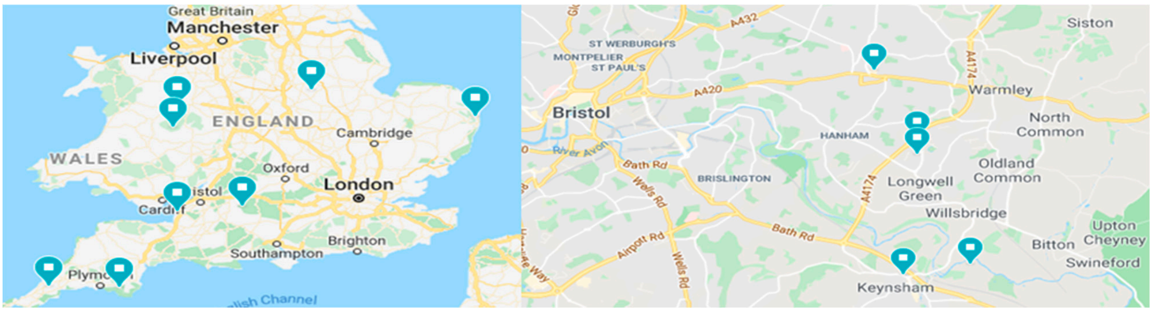

Figure A1. Map of the PFLA farms on the left and the supermarkets on the right [43].

Table A1. Effect of management (conventional, organic and PFLA) and month (April, July and October) on the concentrations of nutritionally relevant FAs in milk. Mean FA proportion ± standard deviation (SD) and ANOVA *p*-values for each FA, expressed as a percentage of the entire FA profile.

	Management			**Month**			**ANOVA *p*-Value** [a]		
	Conventional	**Organic**	**PFLA** [b]	**April**	**July**	**October**	**Man**	**Mon**	**Man × Mon**
Fatty Acid	n = 15	n = 15	n = 15	n = 18	n = 18	n = 18			
C4:0	3.0 ± 0.159	3.1 ± 0.127	3.0 ± 0.221	3.1 ± 0.171	3.1 ± 0.151	3.0 ± 0.235	ns	ns	ns
C5:0	0.13 ± 0.084	0.13 ± 0.124	0.09 ± 0.060	0.11 ± 0.072	0.10 ± 0.055	0.13 ± 0.126	ns	ns	ns
C6:0	2.5 ± 0.096	2.5 ± 0.092	2.5 ± 0.218	2.6 [a] ± 0.154	2.5 [b] ± 0.151	2.5 [b] ± 0.113	ns	***	t
C7:0	0.08 ± 0.066	0.08 ± 0.049	0.06 ± 0.046	0.06 ± 0.051	0.07 ± 0.050	0.08 ± 0.059	ns	ns	ns
C8:0	1.3 ± 0.085	1.3 ± 0.093	1.3 ± 0.144	1.4 [a] ± 0.109	1.3 [b] ± 0.115	1.3 [b] ± 0.078	ns	***	ns
C9:0	0.08 [a] ± 0.041	0.08 [a] ± 0.044	0.06 [b] ± 0.024	0.06 [b] ± 0.030	0.07 [b] ± 0.024	0.09 [a] ± 0.045	*	**	**
C10:0	2.8 ± 0.191	2.7 ± 0.097	2.9 ± 0.495	3.0 [a] ± 0.422	2.7 [b] ± 0.299	2.7 [b] ± 0.241	ns	**	t
C11:0	0.30 ± 0.029	0.31 ± 0.040	0.31 ± 0.054	0.29 ± 0.031	0.31 ± 0.058	0.31 ± 0.040	ns	ns	ns
C12:0	3.5 ± 0.230	3.3 ± 0.146	3.4 ± 0.568	3.6 [a] ± 0.485	3.3 [b] ± 0.344	3.3 [b] ± 0.329	ns	**	ns
C13:0	0.22 ± 0.038	0.23 ± 0.052	0.21 ± 0.050	0.23 ± 0.039	0.20 ± 0.046	0.22 ± 0.054	ns	ns	ns
C14:0	11.0 ± 0.383	11.0 ± 0.325	11.1 ± 1.179	11.2 ± 0.947	11.0 ± 0.887	11.0 ± 0.610	ns	ns	ns
c9 C14:1	1.0 [a] ± 0.069	0.9 [b] ± 0.085	0.9 [b] ± 0.193	0.9 [c] ± 0.148	0.9 [b] ± 0.135	1.0 [a] ± 0.131	**	**	ns
C15:0	1.1 [b] ± 0.062	1.2 [b] ± 0.117	1.4 [a] ± 0.208	1.3 ± 0.242	1.3 ± 0.176	1.3 ± 0.200	***	ns	ns
c9 C15:1	0.05 ± 0.034	0.07 ± 0.050	0.06 ± 0.036	0.06 ± 0.038	0.05 ± 0.029	0.07 ± 0.050	ns	ns	t
C16:0	31.1 [a] ± 1.17	28.6 [b] ± 1.23	28.0 [b] ± 3.92	29.0 ± 3.97	28.6 ± 2.70	29.5 ± 2.29	*	ns	ns
t9 C16:1	0.41 [b] ± 0.091	0.52 [a] ± 0.091	0.59 [a] ± 0.096	0.51 ± 0.118	0.52 ± 0.101	0.52 ± 0.139	**	ns	ns
C16:1	1.9 ± 0.151	1.8 ± 0.146	1.7 ± 0.315	1.7 ± 0.330	1.8 ± 0.163	1.8 ± 0.225	ns	ns	ns
C17:0	0.55 [b] ± 0.080	0.61 [b] ± 0.089	0.68 [a] ± 0.129	0.61 ± 0.122	0.67 ± 0.113	0.59 ± 0.111	**	t	ns
c9 C17:1	0.23 ± 0.045	0.27 ± 0.058	0.29 ± 0.095	0.25 ± 0.057	0.26 ± 0.070	0.30 ± 0.096	t	t	ns
C18:0	9.5 [b] ± 0.619	10.5 [a] ± 0.609	11.2 [a] ± 1.892	10.6 ± 1.708	11.0 ± 1.445	10.1 ± 1.272	**	ns	ns
t6,7,8 C18:1	0.27 ± 0.084	0.30 ± 0.075	0.23 ± 0.123	0.25 ± 0.123	0.25 ± 0.105	0.28 ± 0.082	ns	ns	ns
t9 C18:1	0.20 ± 0.089	0.20 ± 0.086	0.18 ± 0.106	0.17 ± 0.093	0.17 ± 0.097	0.23 ± 0.085	ns	ns	ns
t10 C18:1	0.37 [a] ± 0.099	0.26 [b] ± 0.131	0.19 [b] ± 0.115	0.22 ± 0.125	0.29 ± 0.160	0.26 ± 0.119	**	ns	ns
t11 C18:1	1.1 [c] ± 0.234	1.9 [b] ± 0.284	2.5 [a] ± 1.091	2.0 ± 1.369	1.8 ± 0.601	1.9 ± 0.809	***	ns	ns
t12,13,14 C18:1	0.42 ± 0.065	0.36 ± 0.101	0.32 ± 0.110	0.38 ± 0.097	0.36 ± 0.126	0.35 ± 0.090	t	ns	ns
c9 C18:1	19.0 ± 0.676	19.3 ± 0.752	18.4 ± 2.931	18.5 ± 2.582	19.3 ± 1.898	18.7 ± 1.521	ns	ns	ns
t15 C18:1	0.27 ± 0.094	0.25 ± 0.081	0.25 ± 0.112	0.26 ± 0.110	0.24 ± 0.069	0.27 ± 0.113	ns	ns	*
c11 C18:1	0.53 [a] ± 0.177	0.36 [b] ± 0.155	0.41 [b] ± 0.137	0.38 ± 0.151	0.46 ± 0.176	0.44 ± 0.167	*	ns	ns
c12 C18:1	0.26 [a] ± 0.096	0.17 [b] ± 0.116	0.11 [b] ± 0.064	0.16 ± 0.102	0.18 ± 0.115	0.16 ± 0.111	**	ns	ns
c13 C18:1	0.10 ± 0.064	0.10 ± 0.092	0.10 ± 0.055	0.09 ± 0.070	0.10 ± 0.065	0.11 ± 0.072	ns	ns	ns
c14, t16 C18:1	0.33 ± 0.084	0.35 ± 0.104	0.36 ± 0.120	0.34 ± 0.119	0.35 ± 0.111	0.36 ± 0.089	ns	ns	ns

Table A1. *Cont.*

	Management			Month			ANOVA p-Value [a]		
	Conventional	Organic	PFLA [b]	April	July	October	Man	Mon	Man × Mon
c15 C18:1	0.10 ± 0.044	0.17 ± 0.120	0.13 ± 0.071	0.16 ± 0.111	0.13 ± 0.076	0.12 ± 0.059	ns	ns	ns
t11,15 C18:2 (n3)	0.05 ± 0.041	0.05 ± 0.033	0.05 ± 0.026	0.04 ± 0.026	0.05 ± 0.032	0.05 ± 0.038	ns	ns	ns
t10,14 C18:2	0.06 ± 0.046	0.06 ± 0.046	0.07 ± 0.049	0.05 ± 0.019	0.08 ± 0.048	0.08 ± 0.058	ns	t	ns
c9 t13, C18:2	0.08 ± 0.036	0.09 ± 0.040	0.11 ± 0.070	0.10 ± 0.068	0.09 ± 0.054	0.09 ± 0.043	ns	ns	ns
t9,12 C18:2 (n6)	0.11 ± 0.059	0.10 ± 0.039	0.08 ± 0.047	0.09 ± 0.049	0.09 ± 0.046	0.10 ± 0.056	ns	ns	ns
t8 c13 C18:2	0.14 ± 0.067	0.09 ± 0.057	0.11 ± 0.097	0.09 ± 0.058	0.12 ± 0.070	0.14 ± 0.102	ns	ns	ns
c9 t12 C18:2 (n6)	0.10 ± 0.061	0.11 ± 0.044	0.10 ± 0.066	0.09 ± 0.038	0.11 ± 0.060	0.11 ± 0.072	ns	ns	ns
t9 c12 C18:2 (n6)	0.05 ± 0.033	0.07 ± 0.059	0.06 ± 0.044	0.05 ± 0.041	0.07 ± 0.048	0.06 ± 0.048	ns	ns	*
ctmix10,14,12,16 C18:2	0.09 ± 0.056	0.06 ± 0.030	0.06 ± 0.039	0.07 ± 0.042	0.07 ± 0.047	0.07 ± 0.047	t	ns	ns
t11 c15 C18:2 (n3)	0.16 [b] ± 0.097	0.25 [b] ± 0.112	0.40 [a] ± 0.133	0.27 ± 0.164	0.28 ± 0.156	0.32 ± 0.152	**	ns	ns
c9,12 C18:2 (n6) (LA)	1.8 [a] ± 0.168	1.6 [a] ± 0.232	0.9 [b] ± 0.321	1.4 [a] ± 0.548	1.4 [a] ± 0.430	1.3 [b] ± 0.441	***	*	***
C19:1	0.09 ± 0.033	0.10 ± 0.054	0.10 ± 0.074	0.08 ± 0.029	0.11 ± 0.054	0.11 ± 0.080	ns	ns	t
Unknown LA2	0.09 ± 0.046	0.09 ± 0.052	0.11 ± 0.066	0.08 [b] ± 0.046	0.09 [b] ± 0.047	0.13 [a] ± 0.066	ns	*	ns
Unknown LA3	0.05 ± 0.025	0.08 ± 0.068	0.07 ± 0.068	0.06 ± 0.030	0.08 ± 0.062	0.08 ± 0.077	ns	ns	t
c9,15 C18:2 (n3)	0.03 ± 0.029	0.04 ± 0.024	0.04 ± 0.040	0.03 ± 0.041	0.04 ± 0.030	0.04 ± 0.029	ns	ns	ns
c12,15 C18:2 (n3)	0.05 ± 0.029	0.06 ± 0.045	0.05 ± 0.041	0.05 ± 0.041	0.06 ± 0.043	0.06 ± 0.034	ns	ns	ns
C20:0	0.15 [b] ± 0.034	0.18 [b] ± 0.054	0.22 [a] ± 0.058	0.18 ± 0.046	0.20 ± 0.064	0.18 ± 0.062	**	ns	ns
c6,9,12 C18:3 (n6)	0.04 ± 0.029	0.07 ± 0.046	0.05 ± 0.032	0.04 [b] ± 0.027	0.05 [ab] ± 0.031	0.07 [a] ± 0.043	ns	*	**
c8 C20:1	0.08 ± 0.033	0.11 ± 0.034	0.11 ± 0.038	0.09 ± 0.037	0.10 ± 0.039	0.11 ± 0.034	t	ns	ns
C18:3 (n3) (ALA)	0.47 [c] ± 0.079	0.79 [b] ± 0.125	1.06 [a] ± 0.274	0.78 ± 0.279	0.82 ± 0.331	0.86 ± 0.353	***	ns	ns
c9 t11 C18:2 (CLA9)	0.62 [c] ± 0.071	0.92 [b] ± 0.138	1.16 [a] ± 0.431	0.95 ± 0.502	0.90 ± 0.238	0.98 ± 0.348	***	ns	ns
t11 c13 CLA	0.05 ± 0.031	0.10 ± 0.053	0.09 ± 0.064	0.08 ± 0.062	0.07 ± 0.048	0.09 ± 0.059	t	ns	ns
Unknown CLA1	0.05 ± 0.034	0.08 ± 0.055	0.08 ± 0.054	0.06 ± 0.035	0.07 ± 0.052	0.08 ± 0.062	ns	ns	ns
Unknown CLA2tt	0.07 ± 0.022	0.08 ± 0.052	0.07 ± 0.049	0.07 ± 0.038	0.08 ± 0.041	0.07 ± 0.053	ns	ns	ns
Unknown CLA3tt	0.06 ± 0.043	0.11 ± 0.069	0.11 ± 0.081	0.08 ± 0.069	0.10 ± 0.072	0.10 ± 0.076	ns	ns	ns
Unknown CLA6tt	0.06 ± 0.055	0.07 ± 0.065	0.07 ± 0.065	0.07 ± 0.058	0.06 ± 0.053	0.08 ± 0.072	ns	ns	ns
c9,13,15 C18:3 (n3)	0.05 ± 0.032	0.06 ± 0.027	0.06 ± 0.028	0.05 ± 0.036	0.07 ± 0.020	0.06 ± 0.026	ns	ns	*
c11,14 C20:2 (n6)	0.05 ± 0.028	0.09 ± 0.058	0.06 ± 0.032	0.06 ± 0.024	0.07 ± 0.054	0.07 ± 0.042	ns	ns	ns
c9,11,15 C18:3 (n3)	0.08 [b] ± 0.040	0.13 [a] ± 0.068	0.10 [ab] ± 0.043	0.10 ± 0.047	0.11 ± 0.067	0.10 ± 0.046	*	ns	ns
C22:0	0.11 ± 0.057	0.13 ± 0.019	0.12 ± 0.040	0.12 ± 0.041	0.12 ± 0.031	0.12 ± 0.053	ns	ns	ns
c8,11,14 C20:3 (n6)	0.14 [a] ± 0.052	0.10 [ab] ± 0.030	0.08 [b] ± 0.036	0.10 ± 0.048	0.10 ± 0.040	0.11 ± 0.051	*	ns	ns
c13 C22:1	0.10 ± 0.059	0.08 ± 0.052	0.07 ± 0.047	0.06 ± 0.039	0.09 ± 0.057	0.10 ± 0.054	ns	t	ns

Table A1. *Cont.*

	Management			**Month**			**ANOVA *p*-Value [a]**		
	Conventional	**Organic**	**PFLA [b]**	**April**	**July**	**October**	**Man**	**Mon**	**Man × Mon**
c11,14,17 C20:3 (n3)	0.07 ± 0.061	0.08 ± 0.058	0.07 ± 0.052	0.07 ± 0.046	0.05 ± 0.059	0.09 ± 0.057	ns	ns	ns
c5,8,11,14 C20:4 (n6)	0.21 ± 0.122	0.19 ± 0.090	0.14 ± 0.074	0.17 ± 0.089	0.16 ± 0.096	0.19 ± 0.110	ns	ns	ns
C23:0	0.06 [b] ± 0.033	0.10 [a] ± 0.033	0.08 [b] ± 0.023	0.07 ± 0.038	0.08 ± 0.033	0.08 ± 0.025	*	ns	ns
c13,16 C22:2 (n6)	0.08 ± 0.046	0.10 ± 0.041	0.10 ± 0.030	0.10 ± 0.039	0.08 ± 0.035	0.10 ± 0.039	ns	ns	ns
c5,8,11,14,17 C20:5 (n3) (EPA)	0.09 [b] ± 0.044	0.17 [a] ± 0.089	0.16 [a] ± 0.052	0.14 ± 0.082	0.14 ± 0.070	0.15 ± 0.061	**	ns	ns
C24:0	0.09 ± 0.054	0.15 ± 0.117	0.14 ± 0.094	0.12 ± 0.089	0.14 ± 0.107	0.14 ± 0.088	ns	ns	ns
c15 C24:1	0.06 ± 0.062	0.07 ± 0.042	0.05 ± 0.019	0.06 ± 0.023	0.06 ± 0.057	0.05 ± 0.039	ns	ns	ns
c13,16,19 C22:3 (n3)	0.04 ± 0.029	0.04 ± 0.025	0.04 ± 0.022	0.04 ± 0.028	0.03 ± 0.019	0.04 ± 0.026	ns	ns	*
c7,10,13,16 C22:4 (n6)	0.05 ± 0.024	0.06 ± 0.031	0.05 ± 0.036	0.05 [b] ± 0.023	0.04 [b] ± 0.018	0.07 [a] ± 0.041	ns	**	t
c7,10,13,16,19 C22:5 (n3) (DPA)	0.12 [b] ± 0.030	0.16 [a] ± 0.067	0.18 [a] ± 0.046	0.17 ± 0.057	0.15 ± 0.054	0.15 ± 0.056	**	ns	ns
c4,7,10,13,16,19 C22:5 (n3) (DHA)	0.05 ± 0.062	0.06 ± 0.037	0.04 ± 0.023	0.05 ± 0.034	0.05 ± 0.058	0.05 ± 0.025	ns	ns	ns
SFA [c]	67.7 ± 0.868	66.3 ± 1.487	66.9 ± 4.536	67.7 ± 4.123	66.6 ± 2.948	66.6 ± 2.159	ns	ns	ns
MUFA [d]	27.0 ± 0.800	27.6 ± 0.972	27.1 ± 3.636	26.6 ± 3.353	27.6 ± 2.295	27.3 ± 1.522	ns	ns	ns
PUFA [e]	5.3 ± 0.412	6.2 ± 0.797	6.0 ± 1.522	5.7 ± 1.130	5.8 ± 1.091	6.1 ± 1.261	ns	ns	ns
n-3 [f]	1.2 [c] ± 0.197	1.9 [b] ± 0.374	2.3 [a] ± 0.480	1.8 ± 0.539	1.8 ± 0.590	2.0 ± 0.596	***	ns	ns
n-6 [g]	2.7 [a] ± 0.217	2.4 [a] ± 0.234	1.6 [b] ± 0.455	2.1 ± 0.633	2.1 ± 0.552	2.2 ± 0.573	***	ns	ns
n3n6	0.47 [c] ± 0.083	0.77 [b] ± 0.136	1.40 [a] ± 0.202	0.96 ± 0.488	0.94 ± 0.394	1.01 ± 0.448	***	ns	t
n-6/n-3	2.2 [a] ± 0.377	1.3 [b] ± 0.230	0.7 [c] ± 0.102	1.4 ± 0.727	1.3 ± 0.665	1.2 ± 0.587	***	t	ns
EPA + DPA + DHA	0.25 [b] ± 0.086	0.39 [a] ± 0.147	0.38 [a] ± 0.080	0.36 ± 0.136	0.33 ± 0.119	0.35 ± 0.105	**	ns	ns

Notes: [a–c] Mean values in a row, within management or month, with different letters are significantly different according to Tukey's honestly significant difference test (*p*-values < 0.05). [2] ***: $p < 0.001$, **: $p < 0.01$; *: $p < 0.05$, t: $p < 0.1$, ns: $p > 0.1$. [b] PFLA = Pasture for Life Association, Man = management, Mon = month, SD = standard deviation. [c] Saturated FA (SFA): C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0. [d] Monounsaturated FA (MUFA): c9 C14:1, c9 C15:1, c9 C16:1, t9 C16:1, c9 C17:1, t6,7,8 C18:1, t9 C18:1, t10 C18:1, t11 C18:1, t12,13,14 C18:1, c9 C18:1, t15 C18:1, c11 C18:1, c12 C18:1, c13 C18:1, c14 t16 C18:1, c15 C18:1, C19:1 c8 C20:1, c13 C22:1, c15 C24:1. [e] Polyunsaturated FA (PUFA): t11,15 C18:2 (n3), t10,14 C18:2, c9t13, C18:2, t9,12 C18:2 (n6), t8c13 C18:2, c9t12 C18:2 (n6), t9c12 C18:2 (n6), ct mix 10,14,12,16 C18:2, t11c15 C18:2 (n3), c9,12 C18:2 (n6) (LA), unknown LA2, unknown LA3, c9,15 C18:2 (n3), c12,15 C18:2 (n3), c6,9,12 C18:3 (n6), C18:3 (n3) (ALA), c9t11 C18:2 (CLA9), t11c13 CLA, unknown CLA1, unknown CLA2tt, unknown CLA3tt, unknown CLA6tt, c9,13,15 C18:3 (n3), c11,14 C20:2 (n6), c9,11,15 C18:3 (n3), c8,11,14 C20:3 (n6), c11,14,17 C20:3 (n3), c5,8,11,14 C20:4 (n6), c13,16 C22:2 (n6), c5,8,11,14,17 C20:5 (n3) (EPA), c13,16,19 C22:3 (n3), c7,10,13,16 C22:4 (n6), c7,10,13,16,19 C22:5 (n3) (DPA), c4,7,10,13,16,19 C22:5 (n3) (DHA). [f] Omega-3 FA (n-3): t11,15 C18:2, t11c15 C18:2, c9,15 C18:2, c12,15 C18:2, C18:3 (ALA), c9,13,15 C18:3, c9,11,15 C18:3, c11,14,17 C20:3, c5,8,11,14,17 C20:5 (EPA), c13,16,19 C22:3, c7,10,13,16,19 C22:5 (DPA), c4,7,10,13,16,19 C22:5 (DHA). [g] Omega-6 FA (n-6): t9,12 C18:2, c9 t12 C18:2, t9 c12 C18:2, c9,12 C18:2 (LA), c6,9,12 C18:3, c11,14 C20:2, c8,11,14 C20:3, c5,8,11,14 C20:4, c13,16 C22:2, c7,10,13,16 C22:4.

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