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Milk Fatty Acids: The Impact of Grazing Diverse Pasture and the Potential to Predict Rumen-Derived Methane

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Abstract: The sustainability of dairying has been questioned, yet cattle exploit non-food resources (especially forages) and provide key nutrients for consumers' health. This study, using different forage types, considered milk's nutritional quality, focusing on fatty acid profiles alongside methane emissions—investigating whether methane can be predicted from milk fatty acids (FAs). Compared with grass/clover/maize silage, cows grazing grass/clover pasture produced milk 70% higher in beneficial omega-3 FAs, which increased by an additional 15% when grazing more diverse pasture. Milk from grazing also had less omega-6 FAs (compared with silage diets), and their ratio with omega-3 FAs fell from 2.5:1 on silage to 1.2:1 when grazing grass/clover and 1.1:1 on diverse pasture. Measured methane emissions (at 8.7 g/kg energy-corrected milk) were lower than published values, and existing models for estimating methane from lactating cows were poor predictors for this dataset. The multiple regression of methane against milk FAs in this study provided predictions with an R^2 of 0.56 for daily emissions and 0.65 relative to milk output. Grazing quality and a diverse pasture with productive cows were potentially beneficial to milk nutritional quality, and our results reinforce the theory that milk fat composition could be an accessible tool for methane prediction; however, they also suggest that more work is needed for alternative production systems.

Keywords: dairying; pasture grazing; milk quality; GHG

1. Introduction

Everyone needs to eat, making food production crucial to global sustainability; we need to access nutritious, affordable food without sacrificing the environment or the well-being of producers, consumers, and wild and domesticated animals. The challenge is to identify sustainable production methods or, at the very least, minimise potentially negative influences. Dairy ruminants, consuming fibrous feeds not directly edible by humans, produce high-quality foods and supply key nutrients to many people [1]. This potential for dairy cows to consume waste food, by-product feeds, and widely grown forages means that food choices including some dairy and/or meat can be more sustainable than vegan diets [2]. Nevertheless, the sustainability of animal production has been questioned, due to the potentially negative influences on animal welfare; greenhouse gas emissions (GHG) (particularly methane); biodiversity and eutrophication [3]; consumer health; land-use change; and the inefficient use of human-edible food, especially in systems with high cereal and/or soy use [4]. Science is progressing slowly to mitigate damage, but trade-offs exist between sustainability targets related to economic, environmental, and social elements,

depending on the production systems [3]. For instance, intensification, replacing grazing and other forages with more concentrated, cereal-derived feeds, can reduce the GHG burden per litre of milk [5]. However, this fundamentally changes milk composition to the detriment of fatty acids (FAs), which has repercussions for human health [6,7], and increases the food/feed competition, with greater reliance on potentially human-edible food [8].

Milk and dairy consumption is generally beneficial for health [1], and there is scope for further enriching nutritional quality (and hence social sustainability, via the enhancement of consumer health) by either supplementing dairy cow diets with vegetable oils [9,10] or increasing the proportion of forage eaten, especially through grazing [6,7,11]. Typically, fat consumption in Western diets exceeds nutritional guidelines, and the balance fails to meet the recommended quantity [12]; however, reducing fat intake will exacerbate an already deficient intake of omega-3 polyunsaturated FAs (n-3) [13], unless we change food choices or composition. The World Health Organisation (WHO) [13] guidelines suggest that sustainable diets need to include a higher intake of n-3 but less omega-6 polyunsaturated FAs (n-6) and more polyunsaturated FAs (PUFAs) relative to saturated FAs (SFAs), as well as preformed long-chain n-3 (LCn-3)-like eicosapentaenoic acid (EPA, C20:5); docosapentaenoic acid (DPA, C22:5); and docosahexaenoic acid (DHA, C22:6).

Integrating legume-rich ley forage crops into arable rotations offers numerous sustainability benefits, reducing the need for nitrogen fertilisers (associated with GHG and financial costs) and herbicides, as well as building soil organic matter [14]. With appropriate infrastructure and management, this would also offer a low-cost feed for ruminant livestock. Although alternative forages such as clover [15], alpine pasture [16], and chicory [17] have been shown to enhance milk fat composition, there are no reports on the impact of grazing short-term, diverse lowland pasture on milk fat composition. Since ruminant diets influence the rumen microbiome, they also impact methane emissions, and feed additives, including naturally occurring phyto-metabolites such as tannins [18], and oilseeds [19] have been tested to suppress methane production. If emissions are to be reduced, the impact of dietary changes needs to be assessed; however, measuring or even estimating methane production is far from straightforward [20], and progress would benefit greatly from a reliable proxy indication of methane levels from the rumen.

Since both milk FAs and methane production are influenced by a combination and interaction of ruminants' diets and the rumen microbiome, a promising approach is to investigate if milk fat composition can be used to estimate methane emissions [21,22]. Unfortunately, most findings prove to be specific for each trial and lack repeatability. A more robust prediction method has been derived from a wide-reaching meta-analysis [23] combining data across many studies with a range of feeding strategies—although all appeared to monitor Holstein cows, few considered grazing, and none involved diverse pastures.

This study built on work by Loza et al. [24], considering the role of grazing diverse forage pastures—do they alter (i) the nutritional quality of milk and/or (ii) methane emissions from Jersey cows, compared with grazed grass/clover swards or feeding grass/clover silage? We also considered whether existing prediction models linking milk FAs and methane are appropriate for Jersey cows on alternative forages. Finally, we attempted to identify a feeding management system that reduced methane and enhanced milk FA profiles and assessed whether the latter could be used to estimate the former.

2. Materials and Methods

This paper describes 3 aspects of a trial carried out between February and late May with 24 spring-block-calving organic Jersey cows (calving between February and April) on an organic farm integrating 2-year forage leys into a 4-year arable rotation, linking (i) dietary forage source, (ii) milk fat composition, and iii) methane emissions. Details of the trial site in Northern Germany, procedures for methane quantification using the SF_6 tracer method, and other records collected were reported by Loza et al. [24]. In addition, milk samples, representing 24 h production (from 2x daily milking) were collected, freeze-dried,

and shipped to Newcastle University (UK) for fatty-acid analysis. Methane and milk were collected when cows were fed 1 of 3 forage types: (a) a 50:50 mix of ryegrass/white clover and maize silages consumed in the barn prior to turnout; (b) strip-grazing access to a ryegrass white clover pasture (grass/clover); and (c) strip-grazing access to a diverse pasture sown with ryegrass and white clover plus red clover, plantain, chicory, salad burnet, caraway, and bird's foot trefoil. All forages were supplemented with a concentrate mix of fava beans, triticale, lupins, and maize (9 MJ net energy for lactation (NEL)/kg DM), supplied at 5 kg DM per cow per day whilst housed and reduced to 2 kg DM during the grazing phases. Full details on herd and grazing management and sward composition were provided by Loza et al. [24], although a brief outline is repeated here. During early lactation, prior to turnout, all cows were housed and managed as a single group on silage feeding (11 kg DMI per cow per day of grass/clover and maize silages (6.0 and 6.6 MJ NEL/kg DM, respectively)), but the subsequent grazing phase followed a balanced crossover design on the 2 pasture types. For the initial 5 weeks after turnout (in late March), all cows alternated daily between both pasture types, allowing rumen microbiome adaptation. Then, during the experimental grazing period, cows grazed one of two × 2.2 ha paddocks (on second-year leys, one with grass/clover, the other a diverse sward), divided into daily strips by electric fencing (with back fencing), accessing new areas after morning milking. Daily strips were calculated using measured pre-grazing herbage mass (means for grass/clover and diverse sward, respectively, over the course of the experiment); an allowance of 18 kg DM per cow per day; and target residuals of 4 kg DM. Cows (paired for milk yield, parity, liveweight, and calving date) grazed allocated pastures for 4 days, ahead of 4 days of methane assessment, with milk for FA determination collected on the last day—this pattern was then repeated on the other pasture type. As stated by Loza et al. [24], this relatively short period of adaptation was judged adequate under the circumstances, since all cows (and their rumen microbiota) were exposed to both pasture types for 5 weeks prior to the measurements, and the resulting 8 day period was more than sufficient to stabilise milk fatty acids [25]. Additionally, since concentrate feeding was common in both treatments, the relatively minor adjustments in diet composition from grazing during the 4-day change-over were unlikely to have influenced the rumen microbiota [26]. When methane measurements and milk sampling were conducted in early May, the pre-grazed grass/clover sward averaged 2450 kg DM/ha, with means of 87.1% ryegrass, 12.5% white clover, and 0.3% other plants. The corresponding values for the diverse sward were 2157 kg DM/ha with means of 43.1% ryegrass, 15.0% white clover, 18.0% red clover, 7.0% bird's foot trefoil, 8.6% plantain, 7.5% chicory, and 0.8% other plants (all on a DM basis). On both swards, cows consumed clover and herbs in preference to ryegrass, which represented 96.4% (of the grass/clover) and 74.0% (of the diverse) residual vegetation when cows finished grazing.

2.1. Fatty-Acid Analysis

Milk samples (representing 24 h production) were collected from individual cows on the 4th day of methane assessment, after 7 days on each diet. Samples were freeze-dried prior to methylation and esterification in preparation for gas chromatography/mass spectrometry (GC/MS), using the method described by Stergiadis [27]. The chemicals used for the extraction of FAs, the correction factors for short-chain fatty acids, the analytical standards, and the identification of peaks followed the methodology described by Stergiadis et al. [25].

The GC/MS analysis used an Agilent 8890 gas chromatograph coupled to an Agilent 5977 B MSD (Single Quadrupole Mass Spectrometer). The GC column was a J & W CP-Sil 88 with an injection volume of 1 μL, inlet temperature of 250 °C, and injection mode with a split ratio of 25:1; helium was used as the carrier gas, with a constant flow of 1 mL min^{-1}. The oven temperature programme began at 50 °C for 2 min, increasing to 188 °C at 2.4 °C min^{-1}; remained at this temperature for 40 min; increased to 240 °C at 2 °C min^{-1}; and remained so for 5 min. The MS transfer line temperature was 275 °C. The solvent delay

was 11 min; the ion source was electron impact (EI, 70 eV); the source temperature was 230 °C; and the quadrupole temperature was 150 °C. The total runtime was 130.2 min, and chromatograms were recorded in full-scan mode with a mass range of 50–400 Da.

Fatty-acid concentrations are expressed as % of the whole FA profile. Values for individual FAs were used to calculate total saturated FAs (SFAs), monounsaturated FAs (MUFAs), PUFAs, n-3, n-6, n-3:n-6, n-6:n-3, SFAs:PUFAs, LCn-3 (EPA + DPA + DHA), odd-chain FAs, and an estimate of delta-9 desaturase activity based on C14:1/(C14:0 + C14:1).

2.2. Data Handling and Statistical Analysis

Mixed-effects models were generated using the 'R' package 'nlme' [28] to detect if FA profiles differed between forage types, with Tukey's honestly significant difference test. Records for milk production and composition were used to express methane per kg of energy-corrected (EC) milk, as per Sjaunja [29], as well as total emissions per cow per day. Both values were compared with the FA profile in corresponding milk samples in two ways. Initially, analysis of variance was conducted comparing measured methane against values predicted using models proposed by Bougouin et al. [23]. This recent meta-analysis involved an excess of 800 records collected in 4 countries; however, due to the limitations of the parameters quantified in this study, only some models could be applied to our data. Models 1 (467.3 + 26.7 *C10:0 − 101.1 *[iso C17:0 + trans-9 C16:1] − 122.8 *cis-11 C18:1 − 37.3 *trans-11,cis-15 C18:2) and 6 (372.5 + 18.6 *C10:0 − 79.3 *iso C17:0 + trans-9 C16:1 − 103.4 *cis-11 C18:1 − 38.9 *trans-11,cis-15 C18:2 + 3.6 *milk yield) covered daily output, whereas 15 (13.8 + 9.8 * iso C16:0 − 2.4 * cis-15 C18:1 − 0.4 * [trans-10 + trans-11 C18:1]) and 17 (15.3 + 3.9 * iso C16:0 − 0.5 * [trans-10 + trans-11 C18:1] + 1.9 * milk fat + 2.2 * milk protein − 3.2 * milk lactose) related emissions to milk output. Since ANOVA identified very poor prediction power for these models, the 2nd stage was implemented to quantify correlations between milk FA concentrations and measured methane outputs. Individual FAs, their groups, or ratios with significant Pearson's correlation coefficients ($p < 0.05$) were then used to identify the 'best-fit' multiple regression to predict emissions from fat composition, avoiding those which would over-represent any elements. For example, in calculating total emissions, concentrations of linoleic acid (LA, c9, 12 C18:2) were included, but n-6 or any ratio involving n-6 and LA were not. Additionally, the calculation for the rate of emissions included the ratio of n-3 to n-6 but not the reverse ratio or any of their components (n-3, n-6, or any individual FA, with a strong influence over the total concentrations of these groups). Components with high p-values in initial regressions were eliminated to narrow the gap between R^2 and adjusted R^2 whilst maximising the former. Microsoft Excel was used for data handling, identifying correlations, and multiple regression.

3. Results

All differences in milk composition or its relationship with methane emissions mentioned here or in the discussion were statistically significant ($p < 0.05$), unless stated otherwise. As reported by Loza et al. [24], milk yield and EC milk yield were slightly but significantly higher for the diverse pasture compared with grass/clover (24.9 and 30.3 vs. 23.4 and 29.4 kg/cow/day, respectively), as was the yield of milk fat (1.41 vs. 1.38 kg/cow/day), despite a lower fat content (5.66 vs. 5.92%).

3.1. Forage Type and Milk Fatty-Acid Profiles

The milk FA profiles varied between the three forage types in this study, and the concentrations of individual FAs and their groups or ratios that differed, or are nutritionally relevant, are presented in Table 1, with differences categorised in five ways: (i) For some, we observed a decreasing gradient in concentration when comparing milk from cows grazing the diverse sward, those on the grass/clover sward, and those who fed on silage. In the case of t10 C18:1; vaccenic acid (VA, t11C18:1); conjugated linoleic acid (CLA9, c9t11 C18:2); PUFAs; n-3; and the ratio of n3:n6, cows on the diverse sward produced the highest concentrations, followed by those grazing grass/clover, with milk from silage having the

lowest concentrations or ratios. (ii) The pattern was reversed for n6:n3 and SFAs:PUFAs, which were highest in milk from silage, followed by milk from those grazing grass clover, and lowest for cows on the diverse sward. The concentrations of other FAs, groups, or ratios did not differ between milk from the two grazed swards, regardless of the type, but both were either (iii) lower (ratios of n6:n3 and SFAs:PUFAs) or (iv) higher (t9 C16:1; alpha linolenic acid (ALA, c9, 12, 15 C18:3); DHA; and odd-chain FAs) than milk from housed cows on silage. The other pattern of differences was found for C4:0, EPA, DPA, and LCn-3. Long-chain FAs were generally found in low concentrations, slightly higher in milk from grass/clover grazing (although not always significantly different from the others). In contrast, butyric acid (C4:0) was higher in milk from the diverse swards compared with the grass/clover swards, with neither differing from silage feeding, although the differences were small.

Table 1. Concentrations of individual, nutritionally relevant fatty acids in milk (mean values as % of total fatty acids ± standard error of means) and calculated values which differed between forage types (silage, grass/clover grazing, and diverse sward grazing).

Forage Type	Silage n = 24	Grass/Clover Grazing n = 52	Diverse Sward Grazing n = 58	*p*-Values *
Individual fatty acids				
C4:0	2.2 [ab] ± 0.075	2.0 [b] ± 0.046	2.2 [a] ± 0.054	*
C16.0	31.8 ± 0.401	29.0 ± 0.376	28.5 ± 0.329	t
t9 C16.1	0.32 [b] ± 0.005	0.50 [a] ± 0.008	0.48 [a] ± 0.008	***
C18.0	13.7 ± 0.264	12.5 ± 0.186	12.3 ± 0.218	t
t10 C18:1	0.16 [c] ± 0.005	0.20 [b] ± 0.006	0.23 [a] ± 0.007	***
t11 C18:1 (VA)	0.8 [c] ± 0.039	3.0 [b] ± 0.117	3.4 [a] ± 0.131	***
c9 C18:1 (OA)	18.5 ± 0.395	18.5 ± 0.601	18.3 ± 0.530	ns
c9, 12 C18:2 (LA)	1.9 [a] ± 0.030	1.4 [b] ± 0.025	1.4 [b] ± 0.030	***
c9, 12, 15 C18:3 (ALA)	0.6 [b] ± 0.010	0.9 [a] ± 0.027	1.0 [a] ± 0.032	***
CLA9	0.20 [c] ± 0.010	0.60 [b] ± 0.026	0.71 [a] ± 0.035	***
EPA	0.05 [b] ± 0.002	0.06 [a] ± 0.002	0.05 [b] ± 0.002	*
DPA	0.06 [ab] ± 0.003	0.06 [a] ± 0.002	0.05 [b] ± 0.002	*
DHA	0.000 [b] ± 0.0004	0.006 [a] ± 0.0007	0.003 [a] ± 0.0006	**
Fatty-acid groups and ratios				
SFAs	72.2 ± 0.484	68.5 ± 0.691	68.0 ± 0.543	ns
MUFAs	24.4 ± 0.459	27.3 ± 0.686	27.5 ± 0.550	ns
PUFAs	3.5 [c] ± 0.053	4.2 [b] ± 0.052	4.5 [a] ± 0.069	***
n3	0.9 [c] ± 0.018	1.5 [b] ± 0.029	1.7 [a] ± 0.035	***
n6	2.2 [a] ± 0.034	1.8 [b] ± 0.031	1.8 [b] ± 0.037	***
n3:n6	0.41 [c] ± 0.008	0.84 [b] ± 0.011	0.93 [a] ± 0.012	***
n6:n3	2.5 [a] ± 0.051	1.2 [b] ± 0.015	1.1 [c] ± 0.014	***
EPA + DPA + DHA	0.11 [b] ± 0.005	0.13 [a] ± 0.003	0.11 [b] ± 0.003	**
Odd-chain FAs	2.0 [b] ± 0.024	2.4 [a] ± 0.046	2.4 [a] ± 0.042	*
LA:ALA	3.3 [a] ± 0.070	1.5 [b] ± 0.030	1.4 [b] ± 0.026	***
SFAs:PUFAs	20.9 [a] ± 0.429	16.5 [b] ± 0.287	15.2 [c] ± 0.260	***
d9 activity	4.96 [b] (0.10)	5.99 [a] (0.141)	6.05 [a] (0.149)	***

Values in each row with the same letter do not differ significantly, Tukey's honestly significant difference test (*p*-values < 0.05). *p*-values: *** = $p < 0.001$, ** = $p < 0.01$, *= $p < 0.05$, t = $p < 0.1$, and ns = $p > 0.1$. VA = vaccenic acid; OA = oleic acid; LA = linoleic acid; ALA = alpha linolenic acid; CLA9 = conjugated linoleic acid (c9 t11 C18:2 isomer); EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA=docosahexaenoic acid; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; d9 activity = $100 \times (C14:1/(C14:0 + C14:1))$.

3.2. Relationship between Milk Fatty-Acid Profiles and Methane Emissions

Predictions of the methane output based on milk composition using models from Bougouin et al. [23] had poor agreement with the recorded methane output. For the four models able to be used, the calculated values were always greater than the recorded methane values (Table 2), with all differences being highly significant ($p < 0.0001$).

Table 2. Measured methane output compared with model predictions using models from Bougouin et al. [23] for (a) dairy output (g/cow/day) and (b) relative to milk yield (g/kg energy-corrected milk) (means ± SEM).

(a) Daily Output		(b) Relative to Yield	
Measured	253 ± 9.6	Measured	8.7 ± 0.33
Model 1	476 ± 4.4	Model 15	12.2 ± 0.08
Model 6	365 ± 3.3	Model 17	17.2 ± 0.34

Considering milk FAs against (i) total daily methane emissions (Table 3) and (ii) values expressed relative to milk yield (Table 4) (from within this study) produced very different results, due to the inconsistent relationship between milk composition and methane output (depicted in Figure 1). Only four individual FAs (LA, two minor conjugated C18:2 isomers, and C20:0) and the ratios of LA:ALA and n-6:n-3 correlated significantly in both datasets. The significant positive and negative correlations between the concentrations of individual FAs or calculated values and total methane are shown in Table 3, with a longer list of FAs significantly linked to emissions per kg milk provided in Table 4—in both graphs, significant correlations are indicated in bold. These lists were dominated by minor FAs found in relatively low concentrations, with most appearing to originate in the rumen.

Table 3. Outcome of correlation analysis using fatty-acid profiles (% total fatty acids) and recorded methane emissions (g/cow/day) (showing those with *p*-values < 0.05).

	Pearson's Correlation Coefficient	*p*-Value
Individual fatty acids with negative correlations		
LA (c9, 12 C18:2)	−0.452	**
C20:0	−0.445	**
C20:4 n6	−0.404	*
Individual fatty acids with positive correlations		
CLA3 (t, t)	0.519	***
CLA2 (t, t)	0.484	**
t10 C18:1	0.461	**
t11c15 C18:2	0.426	**
t10t14 C18:2	0.373	*
t11 C18:1	0.336	*
ct mix 10, 14/12, C18:1	0.328	*
c14 + t16 C18:1	0.325	*
Groups or calculated values with negative correlations		
n-6:n-3	−0.506	***
LA:ALA	−0.479	**
n6	−0.381	*
Groups or calculated values with positive correlations		
n-3:n-6	0.482	**

Key: *p*-values—*** = *p* < 0.001, ** = *p* < 0.01, * = *p* < 0.05. Fatty acids and calculated values shown in bold were significantly correlated with both total methane (g/cow/day—Table 3) and milk yield (g/kg energy-corrected milk—Table 4). Fatty-acid abbreviations: LA = linoleic acid, n-6 = omega-6 fatty acids, n-3 = omega-3 fatty acids, ALA = alpha linolenic acid.

Table 4. Outcome of correlation analysis using fatty-acid profiles (% total) and recorded methane emissions relative to yield (g/kg energy-corrected milk) (showing those with *p*-values < 0.05).

	Pearson's Correlation Coefficient	*p*-Value
Individual fatty acids with negative correlations		
c8 C20:1	−0.486	**
t15 C18:1	−0.429	**
C18:0	−0.413	**
C20:0	−0.408	**

Table 4. *Cont.*

Pearson's Correlation Coefficient		*p*-Value
C4:0	−0.407	**
c9 C18:1 (OA)	−0.394	*
c11 C18:1	−0.39	*
C17:1	−0.343	*
LA (c9, 12 C18:2)	−0.314	*
Individual fatty acids with positive correlations		
c15 C18:1	0.327	*
CLA2 (t, t)	0.498	**
C12 + C11:1	0.495	**
C22:2	0.489	**
CLA3 (t, t)	0.477	**
C10:0	0.467	**
t9c12 C18:2	0.452	**
c14 + t16 C18:1	0.450	**
aLN + c11 C20:1	0.443	**
C11:0	0.427	**
C15:1	0.424	**
C14:1	0.400	*
C12:1 + C13:0	0.385	*
C24:1	0.377	*
t11t15 C18:2	0.375	*
C9:0	0.369	*
t9 C16:1	0.362	*
C15:0	0.354	*
c9t12 C18:2	0.336	*
t11c15 C18:2	0.329	*
c15 C18:1	0.327	*
Groups or calculated values with negative correlations		
LA:ALA	−0.540	***
n-6:n-3	−0.521	***
Groups or calculated values with positive correlations		
n-3:n-6	0.507	***
n-3	0.493	**
PUFAs	0.364	*

Key: *p*-values—*** = *p* < 0.001, ** = *p* < 0.01, * = *p* < 0.05. Fatty acids and calculated values shown in bold were significantly correlated with both total methane (g/cow/day)—Table 3) and milk yield (g/kg energy-corrected milk—Table 4). Fatty-acid abbreviations: OA = oleic acid, LA = linoleic acid, n-6 = omega-6 fatty acids, n-3 = omega-3 fatty acids, ALA = alpha linolenic acid.

Multiple regression analysis using the concentration of these significant individual FAs and the two values for methane emissions produced the two potential model equations presented below, with R^2 values of 0.56 and 0.65.

a. Methane (g/cow/day) = 303.8 + (746.9 × [t10 C18:1]) + (687.5 × [c14 + t16 C18:1]) + (4083.3 × [CLA3 (t, t)]) − (362.6 × [t11c15 C18:2]) − (859.8 × [t9t12 C18:2]) − (2085.0 × [ct mix 10,14/12,16]) − (66.9 × ratio n-3/n-6) + (27.0 × [t11 C18:1]) − (488.2 × [t12, 13, 14 C18:1]) − (414.5 × [c12 C18:1]). R^2 = 0.56, adjusted R^2 = 0.40, f = 0.003.

b. Methane (g/kg EC milk) = 4.90 − (126.56 × [c8 C20:1]) − (2.13 × [C4:0]) − (28.36 × [C11:0]) + (2.77 × [C10:0]) + (0.37 × [C18:0]) + (159.51 × [CLA2 (t, t)] + (9.72 × [c14 + t16 C18:1]) − (1.59 × [C12 + C11:1]) + (51.27 × [CLA3 (t, t)]) + (14.59 × [C20:0]) − (0.71 × ratio LA/ALA) + (35.91 × [C22:2]) −(2 5.14 × [t9c12]). R^2 = 0.65, adjusted R^2 = 0.47, f = 0.002.

These equations were used to plot the 40 predicted values against recorded methane emissions, as presented in Figure 2a for total daily methane output and Figure 2b for methane per kg EC milk, with the colour coding indicating the forage type consumed for each datapoint. Three cows were assessed on all three forages; ten on two forages (seven on

both grass/clover and diverse pasture grazing, two on silage and diverse pasture, and one on silage and grass/clover grazing); and the remaining eleven cows were only assessed on a single occasion (one on silage, one on grass/clover grazing, and nine on diverse pasture grazing).
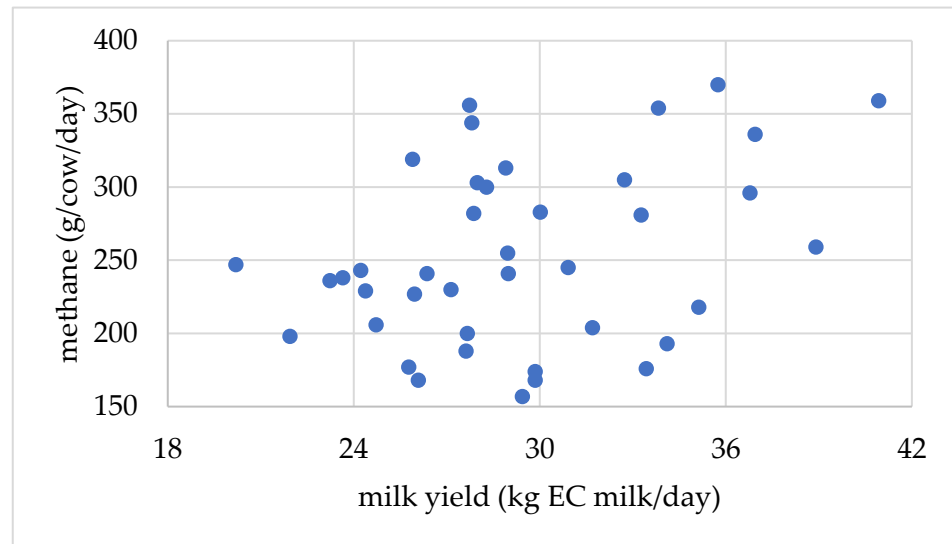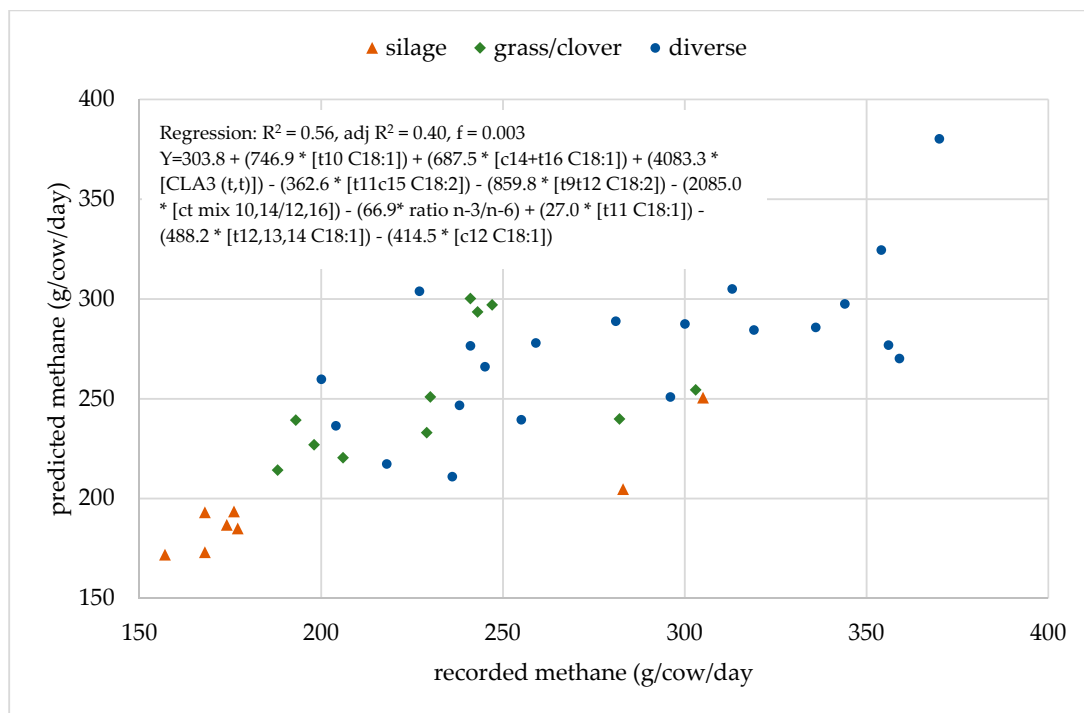

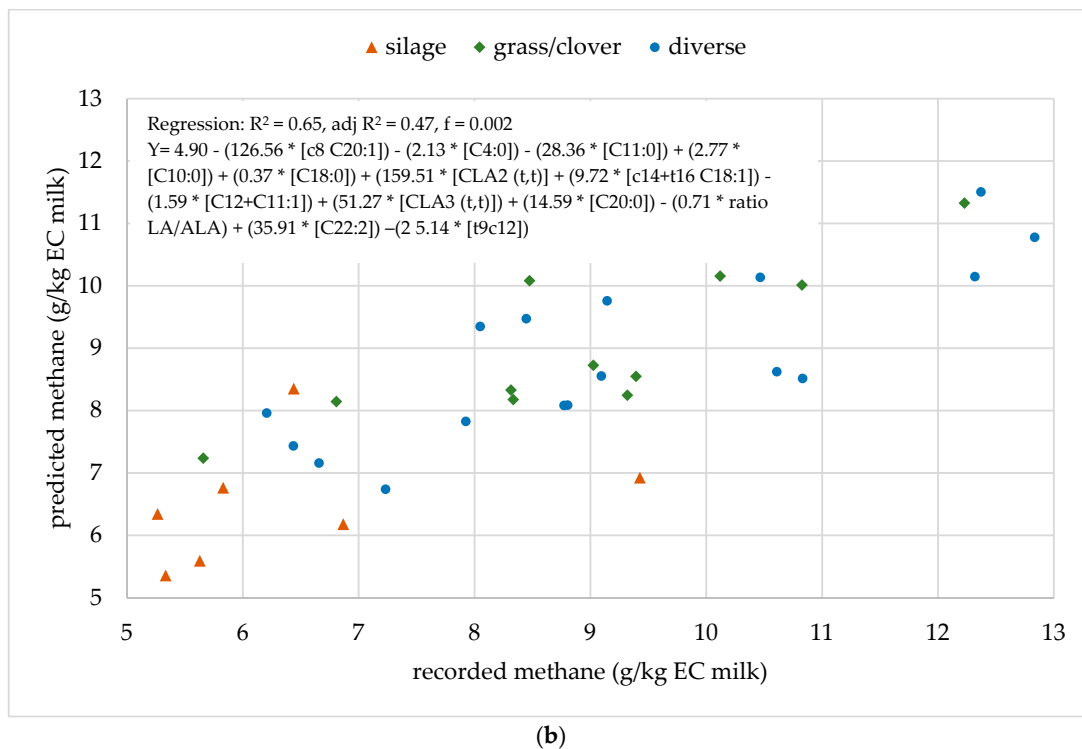**Figure 1.** Relationship between daily methane output and milk yield.

Regression: $R^2 = 0.56$, adj $R^2 = 0.40$, f = 0.003
$Y=303.8 + (746.9 * [t10\ C18:1]) + (687.5 * [c14+t16\ C18:1]) + (4083.3 * [CLA3\ (t,t)]) - (362.6 * [t11c15\ C18:2]) - (859.8 * [t9t12\ C18:2]) - (2085.0 * [ct\ mix\ 10,14/12,16]) - (66.9* ratio\ n-3/n-6) + (27.0 * [t11\ C18:1]) - (488.2 * [t12,13,14\ C18:1]) - (414.5 * [c12\ C18:1])$

(**a**)

Figure 2. *Cont.*

Regression: $R^2 = 0.65$, adj $R^2 = 0.47$, f = 0.002
Y= 4.90 - (126.56 * [c8 C20:1]) - (2.13 * [C4:0]) - (28.36 * [C11:0]) + (2.77 * [C10:0]) + (0.37 * [C18:0]) + (159.51 * [CLA2 (t,t)] + (9.72 * [c14+t16 C18:1]) - (1.59 * [C12+C11:1]) + (51.27 * [CLA3 (t,t)]) + (14.59 * [C20:0]) - (0.71 * ratio LA/ALA) + (35.91 * [C22:2]) −(2 5.14 * [t9c12])

(**b**)

Figure 2. Measured vs. predicted methane output (**a**) per day and (**b**) per kg energy-corrected milk yield, coded according to forage type.

4. Discussion

This study considered the impact of forage type on milk fat composition and the relationship between this and recorded methane emissions.

With respect to fat composition, there is strong past evidence that FA profiles differ between cows fed silage or other conserved forage diets and those with access to grazing [11,30]; therefore, the differences identified in this study between milk produced from housed cows on silage in March and milk from cows grazing in May were not surprising. Hence, this discussion mostly focuses on the differences in the results between the types of grazed swards, providing a novel comparison of milk produced by cows fed on relatively simple grass/clover swards with that of cows fed on more diverse pasture. It has been reported that legume forages (usually red or white clover) enhance the n-3 content of milk (relative to grass only) [10,15,30], and differences in milk composition have been reported from cows grazing diverse alpine [16] or chicory-dominated [17] pastures compared with valley vegetation or ryegrass-dominated swards, respectively. However, it appears that no studies have reported the impact on milk fat composition of introducing greater diversity into short-term grass/clover leys in arable rotations grazed by dairy cows. Soder et al. [31] reported milk fatty-acid profiles from a range of alternative swards, although the high levels of supplements applied (up to 9.2 kg DM/cow/day) and the milk yields from the Holstein cows resulted in quite different baseline/control fat compositions compared to this study. The association between milk FA concentrations and methane emissions identified here is in agreement with many other studies [21–23], though the findings of this study contradicted some existing models, and the differences will be discussed below.

4.1. Comparing Milk Fatty-Acid Profiles in this Study with Other Findings

On the whole, the milk fat compositions recorded herein were comparable with many published reports, dominated by a combination of palmitic acid (PA, C16:0 at 29.8% total FAs) and oleic acid (OA, C18:1 at 18.4% total FAs). The mean concentration of SFAs here (69.6%) was similar to that reported in milk from grazing Jersey cows by

Palladino et al. [32], though slightly lower, and the MUFA (26.4%) and PUFA (4.1%) concentrations were higher than those reported for UK retail Channel Island milk (73% SFAs, 24% MUFAs, and 2.9% PUFAs) [33]. For n-3 and n-6 FA, we also observed differences in relation to other studies. Compared with milk from Jersey cows grazing ryegrass-dominated pasture, the mean ALA and n-3 concentrations were about 40% higher in this study, with LA and n-6 being 3.3 and 2.75 times, respectively, the concentrations reported by Palladino et al. [32]—possibly due to the slightly higher concentrate supplementation in this study (2 kg/cow/day vs. only 0.4 kg). To put this in context, LA concentrations from another study considering diverse forages for Holstein cows (receiving 8–9 kg DM of a maize-based supplements/cow/day) [31] ranged from 4 to 5% of the total FAs—between 2.5 and 3 times higher than the values recorded in this study. Comparing our results with UK retail milk composition [34] confirmed that high-forage diets enhance milk fat composition. Milk from housed cows on grass/clover and maize silages in this study was very similar in LA and n-6 concentrations to 'winter' milk reported by Butler et al. [34], although ALA was 27% higher and LCn-3 eight-fold greater, resulting in lower ratios of LA:ALA and n-6:n-3, possibly due to the influence of clover in the mixed silages in this study. The nutritional advantages offered by high-forage diets appear to be even stronger if milk from grazing cows is compared with the 'summer' milk reported in Butler et al.'s [34] retail study. Combined, the results for milk from grass/clover and diverse swards in this study demonstrated 50% more ALA, almost double the n-3, but approximately 30% less LA and n-6, resulting in a ratio of n-6:n-3 of only 1.1:1, compared with the ratio of 2.6:1 reported for summer milk by Butler et al. [34]. Interestingly, although VA was considerably higher in milk from grazing cows in this study compared with UK summer retail milk [34] (3.2% vs. 1.8%), CLA9 was lower (0.66% vs. 0.81%). This apparent inferior conversion of VA to CLA9 in the udder indicated lower delta-9 desaturase enzyme activity, which has been recognised for Jersey cows (compared with Holstein) [33,35].

4.2. Comparing Milk Fatty-Acid Profiles within This Study

Compared with grass/clover grazing, the diverse swards produced milk higher in many beneficial FAs: VA (+13%), CLA9 (+18%), ALA (+11%), n-3 (+13%), and PUFAs (+7%), all possibly explained by both direct and indirect influences from the alternative plant species consumed. Many plants in the diverse sward (particularly red clover [36] and bird's foot trefoil [37]) were higher in total lipids (especially PUFAs) than grass or white clover, with some also having a higher proportion of n-3 [37]. In addition to enhancing PUFA intake, secondary compounds in many of these plant species also alter rumen lipid metabolism [36], reducing biohydrogenation and allowing more PUFAs to reach the udder and be transferred to milk. Condensed tannins, in plants such as chicory, bird's foot trefoil, and plantain, suppress certain microbial activities, increasing the PUFA content of ruminant milk and meat [38]. The polyphenol oxidase content in red clover has been shown to reduce lipolysis [36,39], again allowing more PUFAs to escape complete hydrogenation, enhancing the amount of MUFAs and PUFAs reaching the udder [40] and potentially allowing CLA9 synthesis from escaping VA [30,41]. The potentially greater PUFA intake from the diverse sward, along with the reduced rumen hydrogenation, could explain the superior milk fat profile.

With respect to these differences in FA profiles between forage types, there were also similarities and differences between our results and those published in other works. However, most other studies have compared alternative vegetation to swards dominated by grasses, as opposed to our study, which assessed the impact of additional diversity compared to a clover-rich sward. The higher concentrations of ALA, n-3, and PUFAs from the diverse pasture in this study (compared with milk from the grass/clover pasture) were in line with many reports considering the impact of 'diverse' forage on milk composition. Stergiadis et al. [15] introduced 20% and 40% (DMI) white clover into a ryegrass/concentrate diet, which elevated ALA by 20% and 43%, n-3 by 12% and 23%, and PUFAs by 10% and 12%, respectively. Comparing milk from cows grazing diverse alpine swards to those on

valley meadows, Leiber et al. [16] found even greater differences for these FAs, with ALA up by 63% and n-3 by 38% from alpine pastures. A similar response has also been reported for cows with access to chicory in addition to grass swards—Mangwe, Bryant, and Gregorini [17] reported 19–26% higher ALA, 17–21% higher n-3, and 10–12% higher PUFAs in cows grazing chicory for 5.5 h each day, and Butler et al. [41] found 10% more ALA in milk from cows on organic farms with clover-based pastures than from comparable low-input herds in areas where inorganic fertiliser created grass-dominated swards. Benchmarking against milk from pastures with a substantial clover content in this study could explain the smaller rise in ALA, n-3, and PUFAs for milk from the diverse pastures (between +7% and 13%) compared to other trials.

Although the changes in n-3 in this study at least partly concurred with previous reports, the changes in the concentrations of CLA9, its precursor VA, LA, and n-6 did not. Herein, the vaccenic acid and CLA9 concentrations were higher in milk from cows on the diverse sward (compared with ryegrass/clover swards), whereas most of the studies mentioned above reported less VA and CLA9 in milk from 'diverse' or alternative forage (compared with grass). Stergiadis et al. [15] reported 8-11% lower concentrations for 40% white clover diets; Leiber et al. [16] 11–12% lower for alpine pasture; Mangwe, Bryant, and Gregorini [17] 15–21% lower after introducing chicory grazing; and Butler et al. [41] 20% lower VA and 29% lower CLA9 in milk from cows on organic farms compared to those on fertilised, grass-dominated swards. The other discrepancy relates to the LA and, hence, n-6 FA concentrations, both of which were stable when cows changed swards in this study, in contrast to reports of higher concentrations in milk from diverse forages: 40% more LA after introducing clover [15], 67% more for alpine pastures [16], 29–34% more for chicory [17], and 24% more (although not a significant difference) for organic compared with fertilised swards [42]. Since both n-3 and n-6 appeared to be elevated for the 'alternative' forages in many of these studies, the authors generally reported no differences in the ratio of n-6:n-3. In contrast, although this ratio in milk from cows grazing grass/clover in this study was substantially lower than that in milk from the housed cows (or the values reported in other published works), it was reduced further still (and significantly) when cows grazed the diverse sward. These highly significant changes in milk fat composition when cows grazed the diverse pasture in this study were all beneficial in terms of consumer health. Even though the milk from our grass/clover pasture had many positive nutritional attributes (compared with other studies), these were enhanced even more when cows grazed diverse pasture, with more ALA, n-3, PUFAs, and CLA9 (and its precursor VA) relative to the less-desirable n-6 FAs and SFAs.

4.3. Predicting Methane Emissions from Fatty Acids

It is reassuring that our results relating milk fat composition to methane emissions reinforced the theory of the link between the two, assuming that both are determined by diet, conditions in the rumen, and associated microbiome activity [21–23]. However, as with the differences discussed for milk fat composition, the relationships identified here for milk FA concentrations and methane output showed similarities and differences with those reported in published works. This was a relatively small dataset (40 records of milk FAs and methane measurements), yet it identified individual milk FAs whose concentrations were significantly related to measured methane output, all be it with very poor alignment to those reported in a large meta-analysis covering many other studies [23]. As in the meta-analysis, 18 of the individual FAs significantly correlating with methane emissions (per kg EC Milk) were known to be of rumen origin—either (i) short-chain FAs synthesised from rumen volatile FAs (e.g., C < 14); (ii) end products (eg C18:0, C20:0) or (iii) intermediates (e.g., t10 and t15 C18:1) of rumen biohydrogenation (and subsequent mammary desaturation (e.g., OA)); or (iv) those with odd-numbered carbon chains. Unfortunately, branched-chain FAs (also of rumen origin) were not quantified in this study and could not be included in our comparison.

Since daily milk yield and methane emissions appeared to be largely independent of each other (Figure 1), it is perhaps not surprising that there was little overlap between the FAs whose concentrations correlated significantly with total emissions and those linked to emissions per kg of EC milk. This was also found in the larger dataset reported by Bougouin et al. [23], where the correlation coefficients between individual FA concentrations differed for methane emissions when expressed as total, per Kg of dry matter intake, or relative to milk yield. Although this meta-analysis dataset, derived from multiple experiments with a wide range of diets, is far more powerful than the dataset included in this study, comparing the results revealed interesting similarities and differences, some of which might need to be explored. There was generally poor agreement between our results and those from the meta-analysis for FAs correlating significantly with total methane emission. Only four individual FAs (t10 C18:1, t11 C18:1, t11c15 C18:2, and LA) were common to both studies (significantly correlating with total methane output), and, except for LA, the positive coefficients herein contradicted the negative relationships in the larger dataset. Compared to these contrasting findings, our −0.45 Pearson's correlation coefficient for LA concentration was not too far from the value of −0.24 reported by Bougouin et al. [23]. For emissions expressed relative to milk output, there was better agreement between our results and those from the meta-analysis, with 12 individual FAs common to both sets of significant correlations, of which six showed comparable positive (C12:0 and C14:1) or negative (OA, LA, c11 and t15 C18:1) Pearson's correlation coefficients.

The methane measurements reported (and discussed) by Loza tended to be lower than those of most other published work, especially for 'intensity' when compared to the high milk output recorded by cows in this study. As such, there were consistent discrepancies between our 40 records when compared to methane predictions from models based on over 800 records in the meta-analysis by Bougouin et al. [23]. Despite ignoring the positive contributions linked to iso-C16:0 (not quantified in our study) from calculations for equations 15 and 17, the predicted methane outputs were all higher than the measured values, by an average of +29–30% for models 6 and 15 and +47 and +49%, respectively, for models 1 and 17. Consequently, stage two was implemented to identify correlating FAs and investigate alternative regression equations that might be more appropriate for this dataset including Jersey cows under organic management, grazing high-quality forages. The resulting multiple regression equations showed reasonable prediction abilities within these experimental conditions, with R^2 values of 0.56 and 0.65. Additionally, the graphs represented in Figure 2 identified outlying records, especially for one cow on the silage diet whose high rate of methane persisted throughout the study. Eliminating this cow's records from the regression calculations enhanced the prediction power, increasing the R^2 values to 0.59 and 0.70—showing the prediction potential of this model if we can identify and remove such inefficient cows from our future dairy herds. Another anomaly that may influence results is the change in body condition during early lactation, since methane tends to be linked to feed intake and rumen activity [23]. If milk produced in early lactation is partially supported by the mobilisation of reserves rather than solely from current intake, this will lower emissions, explaining the clustering of methane recorded for housed cows during early lactation in this study. Interestingly, this is also alters milk fat composition, since the FAs reaching the udder reflect the diet when the reserves were accumulated, as well as the current feeding regimen. The relationship between fat composition and methane proved stronger when methane was expressed relative to milk yield, which is perhaps not surprising, since FA concentrations were expressed relative to total fat and, hence, milk yield rather than as a daily output of each FA.

The strong statistical power of the pooled results in the meta-analysis of Bougouin et al. [23] might make their findings more relevant and reliable than the results of this study; however, since their records were reported solely for Holstein cows, and none of the papers in their analysis considered milk produced on such diverse pastures as reported in this study, some of the discrepancies between our results might be due to the types of cow, forage, or production system considered rather than the weaker statistical power of the current study.

Jersey milk not only contains more total fat and protein than milk from Holstein or Friesian cows, but the composition of the fat differs, with higher concentrations of saturated fatty acids, reducing the relative proportion of unsaturated fatty acids [33]. This shift could alter the relationship between rumen activity, milk FAs, and methane production compared to studies with Holstein or other cows. Considering the individual FAs included in prediction models per litre milk [23], there is a relatively strong negative coefficient applied to t10 and t11 C18:1, and Jersey milk (despite otherwise being lower in unsaturated FAs than other breeds) is significantly higher in VA [33], thus lowering predicted emissions. This is exacerbated when daily yields are apparently elevated by about 20% when calculating EM milk. However, since the impact of dairy diets on methane emissions (from Scandinavian Red cows) has been shown to be stronger than that of subtle variations between individual cows [43], the differences identified here may have been driven by the organic diets and high proportion of clover and other alternative forages consumed in this study, rather than the Jersey genetics. Rumen stoichiometry and the interaction between feed intake (including FA substrates) and rumen microbial activity are highly complex, making the prediction of methane synthesis and the FAs available for secretion into milk challenging. In the quest to achieve a better prediction capability, the findings from this study, considering diverse lowland leys, may have raised more questions than answers.

It is also interesting to compare the findings of this study with the results of Poteko et al. [44], who used linseed to enhance milk fat composition and reduce methane output from housed, silage-fed cows. Although they reported milk FA profiles and methane emissions (assessed in respiration chambers and using a similar tracer method to this study), the authors did not investigate the prediction ability of the later from the former. Oilseeds, especially high-n-3 linseed, are often portrayed as means to improve both the nutritional and environmental sustainability of dairying [9,25,44]. However, our results showed that high grazing intakes of quality grass/clover and diverse pasture by early-lactation Jersey cows produced milk high in beneficial FAs and, as reported by Loza et al. [24], with low methane emissions. Additionally, total milk PUFA levels from the grass/clover or diverse swards in this study were lower than in the linseed study (involving mixed silage diets containing 67 g extruded linseed per kg DM) (4.2 and 4.5 vs. 7.6%), and n-3 was also higher (1.5 and 1.7 vs. 1.3%). In addition, the LA and n-6 concentrations were considerably lower (1.4 vs. 1.9 and 1.8 vs. 2.7%, respectively), resulting in a much lower (i.e., better) n-6:n-3 ratio in milk from cows on grazing diets compared to those fed with a relatively high rate of linseed (1.2 and 1.1:1 vs. 2.1:1). As reported by Loza et al. [24], the methane emissions in this study also appeared to be lower than those reported for the Swiss linseed trial. Over the three diets reported herein, cows in early lactation averaged 253 g methane per day or less than 9 g methane/kg EC milk—38% lower than the 14.1 g/kg EC milk reported for the linseed-supplemented cows by Poteko et al. [44]. Our results indicate that grazing dairy cows on diverse forage swards is beneficial with respect to both the nutritional quality of dairy foods and their environmental impact, adding to other evidence reported for enhanced economic [3], environmental [45], and social sustainability [6,46].

5. Conclusions

This study highlighted the potential nutritional benefits offered by dairy farming in extensive production systems employing grass/clover or diverse forage leys and low concentrate supplementation. On the whole, the levels of beneficial FAs (n-3, PUFAs, and CLA9) in milk were higher than those reported in many other studies, being especially marked when cows grazed the diverse swards. Many beneficial FAs showed a gradient with high levels for the diverse sward, intermediate levels for grazing grass/clover, and the lowest levels for cows fed silages. There were clear relationships between milk FA profiles and methane emissions, and, although the prediction abilities of the equations for this specific dataset might have been relatively weak, they did challenge the application of other prediction models derived in the absence of data from Jersey cows or diverse vegetation.

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