

# Fatty acid profiles and antioxidants of organic and conventional milk from low- and high-input systems during outdoor period

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## Abstract

**BACKGROUND:** Intensification of organic dairy production leads to the question of whether the implementation of intensive feeding incorporating maize silage and concentrates is altering milk quality. Therefore the fatty acid (FA) and antioxidant (AO) profiles of milk on 24 farms divided into four system groups in three replications ( $n = 71$ ) during the outdoor period were analyzed. In this system comparison, a differentiation of the system groups and the effects of the main system factors 'intensification level' (high-input versus low-input) and 'origin' (organic versus conventional) were evaluated in a multivariate statistical approach.

**RESULTS:** Consistent differentiation of milk from the system groups due to feeding-related impacts was possible in general and on the basis of 15 markers. The prediction of the main system factors was based on four or five markers. The prediction of 'intensification level' was based mainly on CLA c9,t11 and C18:1 t11, whereas that of 'origin' was based on n-3 PUFA.

**CONCLUSION:** It was possible to demonstrate consistent differences in the FA and AO profiles of organic and standard conventional milk samples. Highest concentrations of nutritionally beneficial compounds were found in the low-input organic system. Adapted grass-based feeding strategies including pasture offer the potential to produce a distinguishable organic milk product quality.

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**Keywords:** feeding management; organic milk; food quality and health; system comparison; fatty acid profiles

## INTRODUCTION

The characteristic composition and authenticity of organic milk have become important issues of debate, in particular against the backdrop of the emergence of specific consumer expectations and marketing strategies. Differences in the product quality of organic and conventional foods and potential health effects have been discussed controversially during recent years. A consistent analytical differentiation of organic and conventional food has not been recognized yet.<sup>1–3</sup> Although indications of nutritional benefits from the consumption of organic feed are found in animal studies,<sup>4</sup> there are doubts about general health effects of organic food.<sup>5</sup> Unimpressed by the unresolved scientific debate, consumers are buying increasing amounts of organic produce because they suppose organic food to be healthier.<sup>6–8</sup>

As an important part of our Western diet, milk and milk products are investigated in relation to their nutritional benefits. In contrast to their partly negative image, consumption of milk and milk fat shows a negative correlation with asthma and mite allergies in pre-school children,<sup>9</sup> and milk consumption in general cannot be related to a higher risk of cardiovascular diseases.<sup>10</sup> Furthermore, protective effects of farm milk consumption<sup>11</sup> and mothers' consumption of long-chain omega-3 fatty acids (FA), rumen FA such as vaccenic acid (C18:1 t11) and conjugated linoleic acid (CLA) are reported for the development of specific atopic diseases

in breastfed children.<sup>12</sup> A focus of milk quality evaluations is therefore the FA composition, which can be related to several bioactive properties. Apart from the various CLA isomers, mainly CLA c9,t11 and its precursor C18:1 t11, also  $\alpha$ -linolenic acid (C18:3 n-3; ALA), total omega-3 (n-3 polyunsaturated fatty acids (PUFA)) and omega-6 (n-6 PUFA) concentrations and their ratio as well as fat-soluble antioxidants (AO) are discussed.<sup>13–18</sup>

Studies comparing organic and conventional milk quality show differences in terms of higher PUFA, n-3 PUFA and CLA as well as  $\alpha$ -tocopherol and  $\beta$ -carotene concentrations in organic milk<sup>19–21</sup> and establish that milk can be very different in its FA profile composition depending on the production context.<sup>22</sup> A differentiation of organic and conventional milk based on n-3 and

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C-isotopes is suggested by Molkentin and Giesemann.<sup>23,24</sup> It is, however, questionable if an improved FA composition can be related to organic dairy farming *per se*. The FA composition of milk fat depends on a range of factors: season, feeding management and region,<sup>25</sup> overall farm fodder input<sup>20,21</sup> and pasture access.<sup>26</sup> This may explain why, in some regional comparisons, differences between organic and conventional milk samples are not present or are very low.<sup>27–29</sup> Controversial results may also depend on the diverging implementation and strictness of organic regulations and, as one main factor, the orientation of the feeding management at farm level. As an example and direct result of this heterogeneity, milk performance in organic systems ranges from 4000 to 10 000 kg year<sup>-1</sup> per cow.<sup>30,31</sup>

The aim of our system comparison and evaluation at farm level was (1) to assess milk quality and differentiate milk in four different production systems (biodynamic low-input (BLI), biodynamic high-input (BHI), conventional low-input (CLI) and conventional high-input (CHI)), (2) to evaluate the impact of both 'intensification level' (high-input (HI) *versus* low-input (LI)) and 'origin' (biodynamic (B) *versus* conventional (C)) and of the related management factors on milk FA and AO profiles and (3) to reflect the intensification of organic dairy production and implications for organic milk product quality and production.

## MATERIALS AND METHODS

### Selection of dairy farms and grouping of systems

Twenty-four farms were selected, divided into four system groups of six farms each. Selection for B farms was their certification as biodynamic farms by the Demeter Association (Darmstadt, Germany) and for C farms the lack of any organic certification. Biodynamic farms were taken as a subcategory of organic farming (based on EU Regulation No. 834/2007) because of their strict feeding regulations in terms of grazing and concentrate input. Within B farms, low- and high-input feeding strategies were chosen, represented by lower-yielding, grass-based systems (grazed or fresh-cut) and higher-yielding systems, where, in addition to grass, silages of grass/clover and maize and higher amounts of concentrates were fed, respectively. In general, LI farms used no silage throughout the year; hay was fed as sole roughage during winter. Parallel C farms were selected also at two levels of intensification. CLI farms, like BLI farms, were oriented towards feeding of fresh grass only in summer (outdoor period) and hay only in winter (indoor period), supplemented by concentrates in both seasons, whereas CHI farms fed hardly any fresh grass but used silages of grass and maize added with large amounts of concentrates throughout the year. A further prerequisite was a long-standing bulk milk somatic cell count (SCC) below 250 000 cells mL<sup>-1</sup>. The farms were located in the southern part of Germany in the areas Franconia, Hohenlohe, Allgäu and Lake Constance.

### Sampling

Bulk milk samples of at least two milking times were taken in May, July and September 2008. Samples were taken on the same day by three different people of the department staff at all farms and transported at 4 °C in an electric cooling box (Waeco Coolfreeze, Emsdetten, Germany). Samples for the analysis of FA were deep-frozen at -21 °C within 24 h. Fresh milk was delivered to two commercial laboratories within 24 h for the analysis of  $\alpha$ -tocopherol,  $\beta$ -carotene and retinol as well as main milk composition (fat, protein, lactose and SCC), the latter analyzed by

near-infrared spectroscopy. One milk sample was missed on a BLI farm in July, so a total of 71 milk samples were analyzed.

### Feeding ration calculation

On each sampling day the feeding and access to pasture based on farmer information and previous milk control data were recorded. The average daily feed intake per cow was calculated using the program MilliWin 7.0 (Verband Deutscher Ölmühlen e.V., Berlin, Germany). Estimated intakes were calculated using existing lists of fodder analysis of organic feedstuffs from Landesbetrieb Landwirtschaft Hessen (unpublished) and Universität Hohenheim – Dokumentationsstelle<sup>32</sup> and the manufacturer declaration on the composition of concentrates taking into account the milk performance.

### Analysis of fatty acids

The frozen samples were thawed at room temperature and freeze-dried (fd60-1, Pharma & Food, Dresden, Germany). The milk powder was used for Soxhlet extraction with a SOX THERM 2000 S306 A (Gerhardt, Bonn, Germany). Fatty acid methyl esters (FAME) were prepared using NaOCH<sub>3</sub> according to Kramer and Zhou.<sup>33</sup>

Two different gas chromatography (GC) procedures were used to resolve all FA and CLA isomers as described by Kuhnt *et al.*<sup>18</sup> In brief, for the separation of C4 to C22 a fused silica capillary column of medium polarity was used (GC-17 V3, Shimadzu; DB-225MS, 60 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness, Agilent Technologies, Santa Clara, CA, USA). The oven temperature was initially maintained for 2 min at 70 °C, then increased at 10 °C min<sup>-1</sup> to 180 °C, further increased at 2 °C min<sup>-1</sup> to 220 °C and held for 5 min and finally increased at 2 °C min<sup>-1</sup> to 230 °C and held for 27 min. The *cis* and *trans* isomers of C18:1 were separated using a fused silica capillary column of high polarity (GC-2010plus, Shimadzu; CP-select for FAME, 200 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness, Varian, Houten, Netherlands). These isomers were separated under isothermal conditions at 176 °C. For GC analysis, 1  $\mu$ L of 20 g kg<sup>-1</sup> FAME in *n*-hexane was injected with a split ratio of 1:100. For both procedures the injector and detector temperatures were maintained at 260 and 270 °C respectively. The carrier gas was hydrogen. Identification of FA was based on internal standards; for quantification the peak areas were related to the sum of all identified peaks (proportion of FA of total identified FAME).

### Analysis of antioxidants by high-performance liquid chromatography

In a commercial laboratory, hot saponification of the milk was carried out under reflux with ethanolic KOH plus butylated hydroxytoluene as antioxidant following liquid-liquid extraction of the antioxidants with petroleum ether. Chromatographic separations were performed on a LiChrospher RP18 column (5  $\mu$ m, 250 m × 3 mm, Merck, Darmstadt, Germany). The mobile phase consisted of methanol/water (proportion of water from 250 to 0 g kg<sup>-1</sup>) at a flow rate of 1 mL min<sup>-1</sup>. A Merck-Hitachi L-6220 pump and a Spark Basic + autosampler were used (injection volume 25  $\mu$ L). For detection a Jasco UV-2075 with UV detector and a Jasco FP-2020 with fluorescence detector (Jasco UK Ltd, Dunmow, UK) were used. Detection and quantification by peak area and external standards were carried out at the following wavelengths:  $\beta$ -carotene, 456 nm;  $\alpha$ -tocopherol, excitation 295 nm, emission 330 nm; retinol, excitation 325 nm, emission 480 nm. All were done according to

**Table 1.** Location factors, size of farms, milk performance throughout year and milk composition on average during outdoor period of system groups, intensification level and origin

	System group <sup>a</sup>						Intensification level <sup>b</sup>			Origin <sup>c</sup>		
	BLI	BHI	CLI	CHI	<i>P</i> <sup>d</sup>	SEM <sup>e</sup>	LI	HI	<i>P</i>	B	C	<i>P</i>
<i>Farms</i>	<i>n</i> = 6	<i>n</i> = 6	<i>n</i> = 6	<i>n</i> = 6			<i>n</i> = 12	<i>n</i> = 12		<i>n</i> = 12	<i>n</i> = 12	
Altitude (m a.s.l.)	602ab	487b	668a	471b	**	25.8	635	479	**	544	570	0.627
Rainfall (mm year <sup>-1</sup> )	933b	648b	1333a	831b	***	68.3	1133	740	**	791	1083	*
Number of cows	37b	57ab	42ab	68a	*	4.1	40	63	**	47	55	0.343
Holstein Friesian (%)	0	40.1	34.1	61.1	0.109	9.0	17.1	50.6	*	20.1	47.6	0.095
Pasture access (0–1) <sup>f</sup>	1.0a	0.5ab	0.5ab	0.17b	*	0.1	0.75	0.33	*	0.75	0.33	*
Milk yield (kg year <sup>-1</sup> per cow)	4828c	6308b	7335ab	7890a	***	289.4	6082	7099	0.078	5568	7613	***
<i>Milk samples</i>	<i>n</i> = 17	<i>n</i> = 18	<i>n</i> = 18	<i>n</i> = 18			<i>n</i> = 18	<i>n</i> = 18		<i>n</i> = 18	<i>n</i> = 18	
Fat (g kg <sup>-1</sup> ) <sup>g</sup>	39.1	40.0	38.2	40.5	0.196	0.4	38.6	40.3	0.053	39.5	39.4	0.819
Protein (g kg <sup>-1</sup> )	34.4b	32.2c	35.9a	34.0b	***	0.2	35.2	33.1	***	33.3	3.0	***
Lactose (g kg <sup>-1</sup> )	47.6	47.5	47.5	48.1	0.104	0.1	47.6	47.8	0.242	47.6	47.8	0.276
Urea (mg kg <sup>-1</sup> )	238ab	179b	246a	192ab	*	9.0	242	185	**	208	219	0.552
Somatic cell count (10 <sup>3</sup> mL <sup>-1</sup> )	173	184	210	191	0.703	11.0	192	188	0.841	180	200	0.336

Means within a row without a common letter differ at  $P < 0.05$  by Tukey HSD test.

<sup>a</sup> System group: BLI, biodynamic low-input; BHI, biodynamic high-input; CLI, conventional low-input; CHI, conventional high-input.

<sup>b</sup> Intensification level: LI, low-input; HI, high-input.

<sup>c</sup> Origin: B, biodynamic; C, conventional.

<sup>d</sup> *P* value of groups, intensity and origin by one-way ANOVA: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

<sup>e</sup> SEM, standard error of mean.

<sup>f</sup> Pasture access (0–1): 1, pasture access; 0, no pasture access.

<sup>g</sup> Welsh test used in analysis of intensification level.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit<sup>34,35</sup> and Bundesamt für Gesundheit<sup>36</sup> as an in-house method.

### Summed fatty acids and ratios

Over 80 single FA were analyzed (not all listed in Table 3). The choice of presenting a selection of FA, several summed FA and their ratios was based on described indications of these compounds in relation to a specific feeding practice or to nutritional aspects.

### Statistical analysis

#### Multivariate statistics

The identification of potential markers for the differentiation of the system groups and for the prediction of both 'origin' and 'intensification level' with a small number of predictors was based on two different multivariate approaches: permuted stepwise reflected discriminant analysis (RefDA)<sup>37</sup> and permuted stepwise regression (PStR).<sup>38,39</sup> RefDA is a principal component analysis reflected by group information giving reflected components. Stepwise RefDA was computed by a stepwise forward procedure, where variable selection was guided by maximizing the geometric mean reflected variance of the first two or three reflected components. To study the significance of the (stepwise) RefDA solutions, similar random permutation tests were performed as applied in PStR. This method implicitly corrects for multiple hypothesis testing. Since the six measurements throughout the sampling period for each farm might be dependent, we permuted the time measurements within each farm and permuted the farms on a group level. *P* values less than 0.05 were considered significant. Missing data were replaced with the corresponding values from the nearest-neighbor column using Euclidean distances. Data analysis was performed using Matlab software (Version 7.7.0 R2008b, The Mathworks, Natick, MA, USA).

#### Data exploration to differentiate system groups

Several combinations of data sets were used as input for variable selection. The total number of input variables was different per set, and only a limited number of variables were selected with stepwise procedures. A solution with an optimal number of variables was chosen out of the significant stepwise RefDA results, based on the highest cross-validated correct rate of classification (CV-correct). The CV-correct was computed with fivefold cross-validation averaged over 20 replications. The following data sets were analyzed (in parentheses: the total number of input variables; the number of selected variables with the optimal RefDA solution): single FA ( $n = 63$ ; 15), single FA plus AO ( $n = 66$ ; 16), calculated plus summed FA ( $n = 23$ ; 8) and calculated plus summed FA plus AO ( $n = 26$ ; 10).

The analysis with two reflected components showed better stability (always having significant reflected components) than that with three components and therefore only the results based on two components were presented here. The complexity of the different data sets was explored by making bi-plots showing the correlations of the selected data set variables with the reflected components, and in the same figure the mean group scores of the four system groups surrounded by standard deviation (SD) ellipses for each group were also shown. To show the relation with background variables not used for prediction, we made other bi-plots where the correlations from farm and fodder characteristics with the two reflected components were shown together with the previously mentioned SD ellipses for the four system groups.

#### Other statistical analyses

The statistical analyses used in Tables 1–3 were carried out by jmp 8.0 (SAS Institute Inc., Cary, NC, USA). Residuals of the FA and AO variance analysis were tested for normal distribution, with some

**Table 2.** Average estimated composition of feeding ration (kg dry matter (DM) day<sup>-1</sup> per cow) of system groups, intensification level and origin during outdoor period

	System group <sup>a</sup>						Intensification level <sup>b</sup>			Origin <sup>c</sup>		
	BLI	BHI	CLI	CHI	<i>P</i> <sup>d</sup>	SEM <sup>e</sup>	LI	HI	<i>P</i>	B	C	<i>P</i>
<i>Sampling features</i>	<i>n</i> = 17	<i>n</i> = 18	<i>n</i> = 18	<i>n</i> = 18			<i>n</i> = 35	<i>n</i> = 36		<i>n</i> = 35	<i>n</i> = 36	
Grass grazed/cut	12.0a	5.1c	9.3b	1.5d	***	0.59	10.6	3.3	***	8.4	5.4	**
Grass/clover silage	0.0b	5.9a	0.0b	6.1a	***	0.50	0.0	6.0	***	3.0	3.0	1.000
Maize silage	0.0b	1.5b	0.0b	4.0a	***	0.30	0.0	2.8	***	0.8	2.0	*
Hay	1.8ab	1.7b	2.8a	0.3c	***	0.17	2.3	1.0	***	1.7	1.5	0.629
Grass cobs	0.2bc	0.6ab	1.0a	0.0c	***	0.09	0.6	0.3	0.159	0.4	0.5	0.655
Concentrates	0.9c	1.6bc	2.9b	4.5a	***	0.27	1.9	3.1	*	1.2	3.7	***
C/R <sup>f</sup>	6.5c	11.5bc	25.6ab	39.2a	***	2.62	16.3	25.3	0.089	8.9	32.4	***
DM total intake	15.0b	16.4a	16.1ab	16.6a	**	0.19	15.2	16.5	**	15.7	16.3	0.100

Means within a row without a common letter differ at  $P < 0.05$  by Tukey HSD test.

<sup>a</sup> System group: BLI, biodynamic low-input; BHI, biodynamic high-input; CLI, conventional low-input; CHI, conventional high-input.

<sup>b</sup> Intensification level: LI, low-input; HI, high-input.

<sup>c</sup> Origin: B, biodynamic; C, conventional.

<sup>d</sup> *P* value of groups, intensity and origin by one-way ANOVA: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

<sup>e</sup> SEM, standard error of mean.

<sup>f</sup> C/R, concentrate/roughage ratio  $\times 100$ .

data needing to be transformed using Box–Cox transformations. Significant differences were checked with a Bartlett test for variance homogeneity. Differences in the system groups of location and farm data and fodder intake (Tables 1 and 2) as well as FA and AO (Table 3) were subjected to one-way analysis of variance (ANOVA) followed by pairwise comparison with a Tukey honestly significant difference (HSD) test or a Kruskal–Wallis test followed by a Dunn test if variances were unequal. For the Dunn test a Bonferroni correction was used. Means of intensity and origin were tested for significant differences using a *t* test or a Welch test if variances were unequal. The use of specific transformations and statistical tests is highlighted in all tables. Means presented in Tables 1–3 are untransformed original data.

## RESULTS

### Differences between farms and in feeding

Overall location, farm data and fodder intake are summarized in Tables 1 and 2. The four system groups differed as follows. CLI had

the highest rainfall and the farms were located at the highest altitude, while HI farms were located at the lowest altitude. LI farms had the smallest number of cows, followed by BHI, with the highest number found in CHI. C farms achieved the highest milk performance levels, whereas the lowest yields were found in BLI. Milk fat content, lactose and SCC were not different, but protein content was highest in CLI and lowest in BHI; in between were the groups BLI and CHI. Urea levels were highest in CLI and lowest in BHI.

BLI farms used local, dual-purpose breeds (mostly German Brown with different percentages of Brown Swiss). BLI cows grazed day and night and were fed only small amounts of concentrates and some grass cobs, which became visible in an up to twofold higher percentage of both roughage and especially grass-based products in the diet compared with CHI. BHI farms mainly used dual-purpose (German Simmental) and partly milking (Holstein Friesian (HF)) breeds. BHI cows grazed on pastures and/or fed cut fresh grass/clover indoors plus maize and grass silages, concentrates and grass cobs. CLI farms, localized in traditional

**Table 3.** Concentrations of selected fatty acids (mg g<sup>-1</sup> milk fat) and antioxidants ( $\mu\text{g L}^{-1}$ ) in milk from different system groups, intensification level and origin during outdoor period ( $n = 71$ ) and selected summed fatty acids and ratios

	System group <sup>a</sup>						Intensification level <sup>b</sup>			Origin <sup>c</sup>		
	BLI	BHI	CLI	CHI	<i>P</i> <sup>d</sup>	SEM <sup>e</sup>	LI	HI	<i>P</i>	B	C	<i>P</i>
<i>Milk samples</i>	<i>n</i> = 17	<i>n</i> = 18	<i>n</i> = 18	<i>n</i> = 18			<i>n</i> = 35	<i>n</i> = 36		<i>n</i> = 35	<i>n</i> = 36	
Single FA <sup>f</sup>												
10:1	2.99b	2.69b	3.28a	2.86b	***	0.047	3.14	2.78	***	2.84	3.07	*
12:0	32.72ab	32.49b	36.50a	34.12ab	*	0.542	34.67	33.31	0.211	32.61	35.31	*
12:1	0.70b	0.66b	0.86a	0.72b	***	0.016	0.78	0.69	**	0.68	0.79	***
14:0	113.09	113.13	118.89	112.94	0.108	1.035	116.07	113.03	0.144	113.11	115.92	0.177
14:1 c <sup>9</sup>	8.70b	8.78b	10.98a	9.31b	***	0.160	9.87	9.04	*	8.74	10.10	***
15:0 <sup>h</sup>	13.32a	12.64ab	12.18b	11.68b	***	0.160	12.73	12.16	0.067	12.97	11.93	***
16:0	288.63b	315.08a	295.16b	307.69ab	**	2.812	291.99	311.39	***	302.24	301.42	0.886
16:1 c <sup>9</sup>	13.24c	15.72ab	14.62bc	16.34a	***	0.234	13.96	16.03	***	14.52	15.48	*
17:0	6.64a	6.10b	5.54c	5.33c	***	0.746	6.07	5.72	*	6.37	5.43	***
18:0	95.91	92.38	89.11	96.12	0.210	1.355	92.41	94.24	0.501	94.09	92.61	0.588



Table 3. Continued

	System group <sup>a</sup>					Intensification level <sup>b</sup>			Origin <sup>c</sup>			
	BLI	BHI	CLI	CHI	<i>P</i> <sup>d</sup>	SEM <sup>e</sup>	LI	HI	<i>P</i>	B	C	<i>P</i>
18:1 <i>c</i> 9	187.90	187.30	190.20	200.83	0.158	2.407	189.08	194.06	0.303	187.60	195.50	0.101
18:1 <i>c</i> 11	5.49b	5.82b	5.63b	6.92a	**	0.147	5.57	6.36	**	5.65	6.28	*
∑18:1 <i>c</i> 12–15	3.15b	3.82ab	3.58ab	4.00a	*	0.110	3.37	3.91	*	3.49	3.79	0.179
∑18:1 <i>t</i> 4–8	1.48	1.54	1.60	1.61	0.883	0.062	1.55	1.57	0.827	1.51	1.61	0.445
18:1 <i>t</i> 9 <sup>j</sup>	2.23	2.39	2.36	2.46	0.580	0.054	2.30	2.42	0.243	2.31	2.41	0.392
18:1 <i>t</i> 10 <sup>j</sup>	1.69b	2.17b	2.93a	2.55a	**	0.145	2.32	2.36	0.912	1.94	2.74	**
18:1 <i>t</i> 11	24.30a	15.67b	19.76ab	9.37c	***	0.890	21.97	12.52	***	19.86	14.56	**
∑18:1 <i>t</i> 12–16	10.52	10.59	10.43	10.29	0.985	0.276	10.47	10.44	0.952	10.55	10.36	0.732
18:1 <i>c</i> 13	1.05	1.12	1.13	1.04	0.499	0.024	1.09	1.08	0.786	1.09	1.09	0.950
CLA <i>c</i> 9, <i>c</i> 12	11.91	11.63	12.24	13.60	0.211	0.358	12.08	12.61	0.456	11.76	12.92	0.107
CLA <i>c</i> 9, <i>t</i> 11 <sup>j,k</sup>	12.99a	7.93b	12.00a	5.35c	***	0.502	12.48	6.65	***	10.39	8.68	0.088
CLA <i>t</i> 11, <i>c</i> 13 <sup>k</sup>	0.91a	0.72a	0.64a	0.28b	***	0.054	0.77	0.49	*	0.81	0.46	***
CLA <i>t</i> 9, <i>t</i> 11 <sup>i</sup>	0.86	1.07	0.70	0.64	0.094	0.090	0.77	0.85	0.689	0.96	0.67	0.098
CLA <i>t</i> 11, <i>t</i> 13 <sup>i,l</sup>	0.35a	0.25b	0.26b	0.13c	***	0.015	0.31	0.19	***	0.30	0.19	***
18:3 <i>n</i> -3 (ALA)	10.86a	7.04b	7.02b	4.65c	***	0.330	8.89	5.84	***	8.90	5.83	***
20:0 <sup>j</sup>	1.41a	1.47a	1.17b	1.48a	***	0.026	1.29	1.47	***	1.44	1.32	*
20:3 <i>n</i> -6	0.67b	0.75b	0.73b	0.98a	***	0.023	0.70	0.87	***	0.71	0.85	**
20:4 <i>n</i> -6	0.58b	0.64b	0.65b	0.92a	***	0.026	0.62	0.78	***	0.61	0.78	***
20:5 <i>n</i> -3	1.00a	0.80b	0.73b	0.49c	***	0.029	0.86	0.65	***	0.90	0.61	***
22:5 <i>n</i> -3	1.14a	0.96b	0.91b	0.66c	***	0.017	1.02	0.81	***	1.05	0.79	***
Sum FA <sup>m</sup> and ratios												
∑SCFA	76.93	74.84	74.44	74.07	0.163	0.480	75.65	74.46	0.218	75.85	74.26	0.099
∑MCFA	192.11ab	191.35b	207.03a	194.86ab	*	2.106	199.79	193.11	0.113	191.72	200.95	*
∑SFA	686.13	702.17	689.87	697.57	0.279	3.186	688.05	699.87	0.063	694.38	693.72	0.918
∑MUFA	266.28	260.96	269.89	270.22	0.645	2.879	268.14	265.59	0.661	263.54	270.06	0.260
∑PUFA	47.59a	36.88b	40.26b	32.22c	***	0.885	43.82	34.55	***	42.08	36.24	***
∑ <i>n</i> -3 PUFA	16.64a	12.18b	11.89b	8.79c	***	0.405	14.19	10.49	***	14.35	10.34	***
∑ <i>n</i> -6 PUFA <sup>i</sup>	20.42b	20.62b	20.00b	23.61a	**	0.545	20.21	22.11	0.082	20.52	21.80	0.243
∑CLA	16.61a	11.29b	14.91a	7.37c	***	0.601	15.73	9.33	***	13.87	11.14	*
∑OBCFA <sup>i</sup>	41.81a	37.81bc	37.02b	34.99c	***	0.295	39.35	36.40	***	39.75	36.01	***
∑C18:1( <i>t</i> )	40.23a	32.35b	37.08ab	26.28c	***	0.985	38.61	29.32	***	36.18	29.32	*
PUFA/SFA	0.07a	0.05bc	0.06b	0.05c	***	0.001	0.06	0.05	***	0.06	0.05	**
<i>n</i> -6/ <i>n</i> -3 <sup>j</sup>	1.25c	1.78b	1.71b	2.80a	***	0.097	1.48	2.29	***	1.52	2.26	***
18:1 <i>t</i> 10/ <i>t</i> 11 <sup>i</sup>	0.07a	0.18b	0.16b	0.37c	***	0.020	0.13	0.24	***	0.13	0.24	***
16:0/18:2 <i>n</i> -6	19.92b	26.59a	22.67ab	20.29ab	*	0.896	21.33	23.44	0.243	23.35	21.48	0.300
18:1 <i>c</i> 9/18:0	1.97b	2.04ab	2.14a	2.11ab	*	0.022	2.05	2.07	0.650	2.01	2.12	**
16:1 <i>c</i> 9/16:0	0.05b	0.05ab	0.05ab	0.05a	**	0.001	0.05	0.05	*	0.05	0.05	*
18:1 <i>t</i> 11 + CLA <i>c</i> 9, <i>t</i> 11	37.29a	23.60b	31.76a	14.72c	***	1.368	34.44	19.16	***	30.25	23.24	*
Antioxidants												
α-Tocopherol <sup>l</sup>	985a	832ab	695b	658b	***	30.019	836	745	0.132	906	676	***
β-Carotene	159a	136ab	120bc	99c	***	4.680	139	117	*	148	109	***
Retinol	343	374	394	388	0.140	8.281	369	381	0.489	359	391	0.054

Means within a row without a common letter differ at  $P < 0.05$  by Tukey HSD test.

<sup>a</sup> System group: BLI, biodynamic low-input; BHI, biodynamic high-input; CLI, conventional low-input; CHI, conventional high-input.

<sup>b</sup> Intensification level: LI, low-input; HI, high-input.

<sup>c</sup> Origin: B, biodynamic; C, conventional.

<sup>d</sup>  $P$  value of groups, intensity and origin by one-way ANOVA or (<sup>i</sup>) by Kruskal–Wallis test: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

<sup>e</sup> SEM, standard error of mean.

<sup>f</sup> Single FA, single fatty acids: *t*, *trans*; *c*, *cis*; ALA, α-linolenic acid.

<sup>g</sup> Welsh test used in analysis of intensification level.

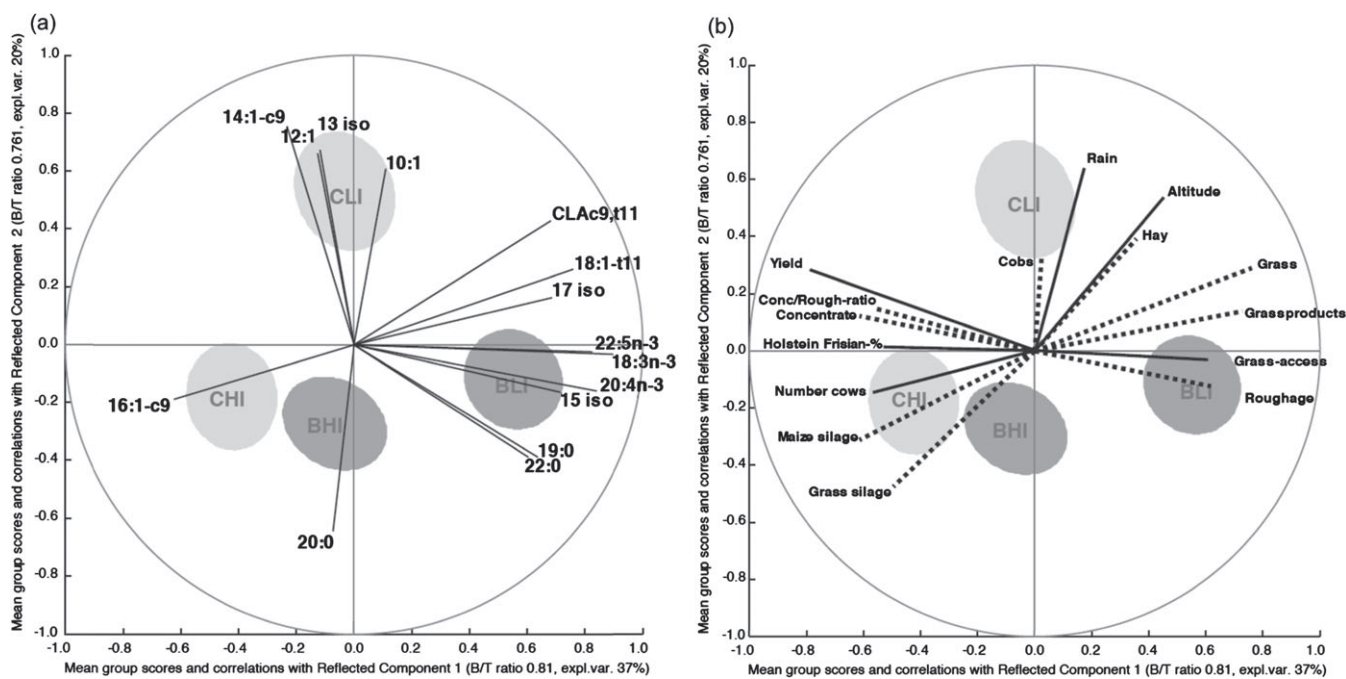
<sup>h</sup> Dunn test and Bonferroni correction used in analysis of system groups.

<sup>j</sup> CLA *c*9,*t*11: coeluted with CLA *t*8,*c*10 and CLA *t*7,*c*9.

<sup>k</sup> Box–Cox transformed data for analysis of system groups; means presented are untransformed original data.

<sup>l</sup> Welsh test used in analysis of origin.

<sup>m</sup> Sum FA, summed fatty acids: SCFA, short-chain fatty acids (C4–C8); MCFA, medium-chain fatty acids (C10–C14); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; OBCFA, odd-branched-chain fatty acids; *n*-3 and *n*-6 PUFA, omega-3 and omega-6 fatty acids respectively.



**Figure 1.** Differentiation of milk from four system groups in a reflected discriminant analysis (RefDA) visualized in bi-plots of group means of reflected components with corresponding standard deviation ellipses and correlations with (a) single fatty acids (FA) and (b) farm factors and feeding management. Both (a) and (b) show results for two reflected components of the same optimal RefDA solution, where 15 single FA out of 63 were selected with stepwise RefDA (CV-correct = 0.75, *P* values = 0.002 and 0.001). Abbreviations: B/T, between-group/total variance ratio for reflected component; expl. var., percentage of variance of selected FA explained by reflected component; BLI, biodynamic low-input; BHI, biodynamic high-input; CLI, conventional low-input; CHI, conventional high-input; Conc/Rough-ratio, concentrate/roughage ratio; CV-correct, cross-validated correct rate of classification computed with fivefold cross-validation averaged over 20 replications.

grassland regions, used local, dual-purpose and/or milking breeds (German Brown, Brown Swiss or HF). Some of those farms practiced pasturing several hours a day, but all farms fed fresh-cut grass indoors daily. In comparison with BLI, higher levels of concentrates and grass cobs were fed in CLI. CHI cows were fed indoor total mixed ration (TMR) with high proportions of maize and grass silages plus concentrates, while the proportion of fresh grass products as well as total roughage was lowest and, in contrast to all other systems, only lowest amounts of hay were fed (Table 2). CHI farms used more milk-oriented breeds (HF and some German Simmental).

**Differences in FA and AO profiles**

*FA profiles (Table 3)*

A general pattern often found was that BLI and CHI showed the largest contrast and BHI and CLI were in an intermediate position. BLI most often showed the highest concentrations for PUFA, n-3 PUFA, CLA (together with CLI), odd-branched-chain fatty acids (OBCFA) and total C18:1 and was also highest in the ratios PUFA/saturated fatty acids (SFA) and C18:1 t10/C18:1 t11 and in C18:1 t11 + CLA c9,t11 (together with CLI). The lowest ratios in BLI were found for n-6/n-3, C18:1 t9/t11, C16:0/C18:2 n-6, C18:1 c9/C18:0 and C16:1 c9/C16:0. In contrast, the lowest concentrations in CHI were found for PUFA, n-3 PUFA, CLA, OBCFA and total C18:1, and CHI was also lowest in the ratios PUFA/SFA and C18:1 t10/C18:1 t11 and in C18:1 t11 + CLA c9,t11. CHI showed the highest concentrations for n-6 PUFA and was highest in the ratios n-6/n-3 and C16:1 c9/C16:0. CLI showed the highest concentrations for medium-chain fatty acids (MCFA), CLA and C18:1 t11 + CLA c9,t11 (together with BLI) and was highest in the ratio C18:1 c9/C18:0.

Individual FA showed several pronounced differences for all system groups. This was prominently found for C15:0, C17:0, C18:1 t11, CLA c9,t11, CLA t11,c13, CLA t11,t13, ALA, C20:0, C20:5 n-3 and C22:5 n-3 and in inverse proportion for C16:1 c9, ΣC18:1 c12–15, C18:1 t10, C20:3 n-6 and C20:4 n-6. Several other single FA showed only small differences or none, as listed in Table 3.

For the factor ‘intensification level’ the highest levels of significance (\*\*\*) were found in summed FA for LI in PUFA, n-3 PUFA, CLA, OBCFA and total C18:1 and were also highest in the ratio PUFA/SFA and in C18:1 t11 + CLA c9,t11 and lowest in the ratios n-6/n-3, C18:1 t10/C18:1 t11 and C16:1 c9/C16:0.

For the factor ‘origin’ the highest levels of significance (\*\*\*) were determined in summed FA for B in PUFA, n-3 PUFA, CLA, OBCFA and total C18:1 and were also highest in the ratio C16:1 c9/C16:0 and in C18:1 t11 + CLA c9,t11 and lowest in MCFA and the ratios n-6/n-3, C18:1 t10/C18:1 t11 and C18:1 c9/C18:0.

*AO profiles (Table 3)*

The highest levels of α-tocopherol were found in B milks. BHI was similar to C, whereas BLI showed the highest level. β-Carotene was highest in B and lowest in CHI, while CLI was similar to BLI and CHI. No differences could be detected for retinol.

**Differentiation of system groups by RefDA**

A differentiation of the milk FA complexity between the four groups was possible based on 15 specific FA markers (Fig. 1(a)). The differentiation was achieved with a CV-correct of 0.75 and *P* values for the two reflected components of 0.002 and 0.001 respectively. On the first axis, BLI and CHI could be differentiated, while BHI and CLI showed an intermediate and overlapping position that could

**Table 4.** Prediction models of factors 'origin' and 'intensification level' based on permuted stepwise regression (PStR). Models shown were based on (1) single fatty acids, (2) summed and calculated fatty acids, (3) single fatty acids plus antioxidants and (4) summed and calculated fatty acids plus antioxidants

Variant	Fatty acids	$R^2$ <sup>a</sup>	$P$ <sup>b</sup>	CVC <sup>c</sup>	NrV <sup>d</sup>	Predictive variables <sup>e</sup>				
						1st	2nd	3rd	4th	5th
Origin	(1) Single	0.69	0.003	0.90	5	22:5 n-3	14:1 c9	18:1 c9	14:0	18:1 c13
	(2) Sum <sup>f</sup>	0.60	0.006	0.81	5	$\sum$ n-3	$\sum$ CLA	18:1 t10/t11	18:1 c12-15	MUFA
	(3) Single + AO <sup>g</sup>	0.65	0.006	0.88	4	22:5 n-3	14:1 c9	$\alpha$ -Tocopherol	18:1 c9	
	(4) Sum + AO	0.58	0.009	0.79	5	$\sum$ n-3	$\sum$ CLA	18:1 t10/t11	$\beta$ -Carotene	Retinol
Intensification level	(1) Single	0.71	0.001	0.95	4	RA	18:2 t11,c13	ALA	14:1 c9	
	(2) Sum	0.59	0.001	0.83	4	VA + RA	$\sum$ PUFA	$\sum$ MCFA	$\sum$ CLA(t,t)	
No differences if AO were included										

<sup>a</sup>  $R^2$  = coefficient of determination.  
<sup>b</sup>  $P$  value.  
<sup>c</sup> CVC, cross-validated correct rate of classification.  
<sup>d</sup> NrV, number of variables.  
<sup>e</sup> Predictive variables: n-3, n-3 PUFA (omega-3 fatty acids); RA, rumenic acid (CLA c9,t11); VA, vaccenic acid (C18:1 t11); c, cis; CLA, conjugated linoleic acid; t, trans; PUFA, polyunsaturated fatty acids; ALA,  $\alpha$ -linolenic acid; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids.  
<sup>f</sup> Sum, summed fatty acid groups.  
<sup>g</sup> AO, antioxidants.

be differentiated on the second axis. In the bi-plot of Fig. 1(a) the mean group scores of the four system groups surrounded by SD ellipses for each group are presented. BLI was characterized by higher portions of CLA c9,t11, C18:1 t11, C17iso, C22:5 n-3, ALA, C22:0, C20:4 n-3, C15iso and C19:0 and lower portions of C16:1 c9, while CHI was characterized vice versa. CLI was characterized by higher portions of C14:1 c9, C13iso, C12:1 and C10:1 and lower portions of C20:0, while BHI was characterized vice versa. In a bi-plot, correlations with management factors and farm features (Fig. 1(b)) were computed with the reflected components of the selected 15 differentiating single FA (Fig. 1(a)) in the milk from the four system groups.

Based on the correlations of feeding factors with the two reflected components, a characterization of the system groups was possible. First, in regard to the feeding regime, the system separation was obtained by a high proportion of fresh grass and grass products and an overall high level of roughage at BLI farms, whereas CHI was correlated with a high concentrate level, a high concentrate/roughage ratio and a high level of maize and grass silages (first axis in Fig. 1(b)). On the second axis, which separated CLI and BHI, the high proportion of hay and grass cobs was correlated with CLI, whereas the higher use of silages was connected with BHI. The main diverging system management patterns were characterized, on the one hand, by a correlation between full pasture access for BLI and dual-purpose cows and, on the other hand, for CHI farms, by high-yielding cows that were indoor-fed and with a high proportion of HF. The first axis in Fig. 1(b) indicated the changes in the cows' diet from fresh grass towards maize and grass silage and higher concentrate inputs.

### Prediction of origin and intensity by PStR

It was possible to predict both 'origin' and 'intensification level' on the basis of only four or five variables (Table 4). The prediction level was high, with a coefficient of determination ( $R^2$ ) of 0.58–0.71, a  $P$  value of 0.001–0.009 and a CV-correct of 0.79–0.95. In all models predicting 'origin', the first predictive variables were n-3 FA (total n-3 PUFA or C22:5 n-3), whereas, in models predicting 'intensification level', CLA c9,t11 (because of space reasons in

Table 4 named RA (rumenic acid)), either alone or in combination with its precursor C18:1 t11 (in Table 4 named VA (vaccenic acid)), was the first predictor.

## DISCUSSION

Our system comparison offered the possibility to evaluate the main impacts of a system intensification based on the incorporation of maize and grass silages plus concentrates at the expense of fresh grass, and vice versa, within organic and conventional dairy production at farm level. In contrast to a factorial feeding trial, the system groups had simultaneous changes of multiple and partly overlapping system factors.

### Impact of system feeding and basic farm factors on FA profiles

#### Feeding factors

As shown by the bi-plots in Fig. 1, the main separation in feeding management between the system groups was due to the different amounts of fresh grass, maize and grass silage and concentrate intake between BLI and CHI. Differences in the FA profiles of milk reflect different uptake levels in the concentrations of ALA in forages, differences in bypass and escape of FA in the rumen as well as rumen bio-hydrogenation levels. The intake of ALA is highest from fresh, fast-growing grass and therefore a decrease in ALA in milk occurs if grass is replaced by conserved forages or by concentrates.<sup>40</sup> Maize silage negatively affects the FA composition in terms of long-chain PUFA, n-3 PUFA and OBCFA and total *trans* fatty acids (TFA).<sup>41,42</sup> In contrast to a grass silage-based ration, the n-6/n-3 ratio in the milk of maize silage-fed cows will increase as well in our B as in our C system group (Table 3). Chilliard *et al.*<sup>43</sup> show an increase in C18:0, C18:1 c9, ALA and CLA when animals are on pasture, whereas levels of C10:0–C16:0 decrease. Several indications of a gradual change between the four system groups could be shown for the ratio C18:1 t10/C18:1 t11, ALA, CLA c9,t11 and CLA t11,c13 (also high in CLI), with highest levels in BLI through intermediate levels in BHI and CLI to lowest levels in CHI, contrasted by the highest n-6/n-3 ratio level in CHI to the lowest in BLI.

The FA profile of BLI was characterized by a range of n-3 PUFA: ALA, C20:4 n-3 and C22:5 n-3. These long-chain n-3 PUFA could only be synthesized from ALA in low amounts by  $\Delta 5$ - and  $\Delta 6$ -desaturase.

CLA c9,t11 as the main isomer of CLA also showed this common pattern. CLA in milk is synthesized in the rumen from linoleic acid, while the majority of CLA c9,t11 is synthesized endogenously by  $\Delta 9$ -desaturase from the conversion of C18:1 t11 in the mammary gland.<sup>44</sup> High CLA levels are positively correlated with the summer season<sup>40,45</sup> and are effected through grass intake and grazing intensity<sup>46</sup> and negatively correlated with the feeding of maize silages<sup>41</sup> or concentrates.<sup>47</sup> CLA c9,t11 and CLA t11,c13 are discussed as important indicators for the grass share in the ration as well as for alpine or organic origin of the milk.<sup>13,48</sup> Vlaeminck *et al.*<sup>49</sup> review the qualitative changes in OBCFA. It is shown that an increase in odd iso-FA is found when diets increase the amount of forage in relation to concentrates. Changes are related to an increase in activity of rumen cellulolytic bacteria and not of amylolytic bacteria. The discriminating iso-FA found in BLI milk (Table 3) fitted very well with these findings.

#### *Other basic farm factors*

These factors, highlighted in Fig. 1(b), had no, only limited or just an indirect impact on the FA composition of milk. Among them, the number of cows or even the percentage of HF in the herds could be regarded as less relevant, since the impact of breed<sup>43</sup> or altitude<sup>50</sup> can be regarded as secondary. Nevertheless, those factors characterized the system CHI as a common modern dairy production in which high-performing HF breeds with high milk performances that need to be fed high-energy rations incorporating high levels of concentrates and maize are preferred. The second axis in Fig. 1(b) showed also the correlation of CLI with higher rainfall and with the increased height above sea level of these farms. Rainfall and altitude, as environmental factors, which were highest in LI systems (Table 1), had an indirect impact on feeding management by governing the particular system orientations and practices. Farms in these regions were more suitable for grassland and traditionally practiced dairy production. Hay-drying facilities were commonly present and LI farms also incorporated the highest proportions of grass cobs in the ration.

### **Differentiation of milk**

#### *Differentiation of milk from system groups*

Both B milks from either low- or high-input systems were characterized by consistently different FA profiles compared with CHI milk. CHI could be taken as representative of modern conventional dairy production, and most milk sold in German supermarkets is being produced under quite similar conditions. The ALA and CLA c9,t11 levels of milk from conventional systems reported by Kraft *et al.*<sup>13</sup> (in conventional milk: ALA 3.3 mg g<sup>-1</sup> fat and CLA c9,t11 2.8 mg g<sup>-1</sup> fat) and Butler *et al.*<sup>20</sup> (in conventional milk from Italy, Sweden, Denmark and Great Britain: ALA 3–6 mg g<sup>-1</sup> fat and CLA c9,t11 4–7 mg g<sup>-1</sup> fat) are comparable to the levels found in CHI. In addition, the farm size, the preference for using milk-oriented HF breeds, the feeding management of TMR as well as the milk performance level were all in accordance with standard practices in conventional German dairy production.<sup>51</sup>

BLI milk showed the typical composition of a grass-based system, as described beforehand, while CHI milk was typical for a TMR-based high-input system. CLA t11,c13 showed nearly twofold higher levels in B milks and more than threefold higher levels in BLI

compared with CHI. This isomer is proposed as a marker for organic milk by Kraft *et al.*,<sup>13</sup> and already in 1997 the use of OBCFA in the differentiation of organic from conventional milk was suggested by Jahreis *et al.*<sup>52</sup> The gradual decrease in this isomer from highest levels in BLI through BHI and CLI to CHI (Table 3) underlined the effects of the feeding of grass and crude fiber, which were highest in BLI, compared with a ration high in starch, as practiced in CHI, on the activity of rumen bacteria,<sup>49</sup> and those former assumptions of certain OBCFA as important markers of milk origin could be confirmed.

Anyhow, a resemblance of the milk of system groups BHI and CLI could also be detected in the statistical evaluation, which showed similar levels of selected relevant indicators such as PUFA and ALA but differences in CLA c9,t11 and C18:1 t11 (Table 3). CLA c9,t11 and C18:1 t11 were higher in the milk of CLI systems. The effect of maize and concentrates on the reduction of CLA c9,t11 and C18:1 t11 levels in milk due to changes in ruminal pH and bio-hydrogenation level has been shown.<sup>41</sup> The ALA levels in milk are correlated with the intake of grass.<sup>53</sup> BHI and CLI had nearly the same share of grass in the ration, whether fresh or conserved, but higher levels of maize silage were used in BHI or higher levels of concentrates in CLI, which both decreased the ALA levels in milk compared with BLI.

#### *Differentiation of 'intensification level' and 'origin' by multivariate statistical approaches*

Both factors could consistently be differentiated. The prediction could be based on two main marker groups (Table 4): for 'intensification level', mainly CLA isomers and their precursors were responsible, while 'origin' was characterized by n-3 PUFA. The differentiation of organic milk based on higher levels of n-3 PUFA is also shown by Molkentin,<sup>54</sup> who included delta C-isotopes in his prediction. Concentrations of CLA, especially CLA c9,t11 and CLA t11,c13, and also C18:1 t11 in milk sensitively reflected the proportion of fresh grass and the abundance of maize in the cows' ration. Previously, CLA t11,c13 and CLA c9,t11 were proposed as markers of alpine and organic origin.<sup>13</sup> The 'intensification level', mainly reflected by the share of fresh grass in the ration, which was up to 10.6 kg dry matter (DM) day<sup>-1</sup> per cow in LI, and the renunciation of maize and silages in the ration, was the most important factor impacting the FA profile of the milk. Desired FA (CLA c9,t11 and n-3 PUFA) were also found in higher levels in systems that were pasture based on permanent grassland.<sup>53</sup> To the highest extent, this characteristic was reflected in the combination of factors B and LI.

#### *TFA in milk as a relevant factor of nutritional importance*

Total TFA are controversially discussed in relation to their health effects. They were main indicators for system differentiation: higher levels of total TFA were found in LI and B systems. Here, C18:1 t11 was the C18:1 *trans* isomer found in highest proportion, with up to 24.30 mg g<sup>-1</sup> milk fat in BLI (Table 3). TFA profiles can differ; while industrially derived fats are characterized through mainly t9 and t10 isomers, ruminant TFA are mainly t11. The formation of the t11 double bond in the rumen appears to be unique. Only C18:1 t11 can be transformed into CLA c9,t11 by  $\Delta 9$ -desaturase in humans.<sup>18</sup> In the present study, C18:1 t11 levels were more than twofold higher in LI systems compared with CHI, with relatively highest levels in BLI, whereas levels of 18:1 t10 were highest in both C systems and cannot be further transformed into CLA owing to the lack of  $\Delta 12$ -desaturase in mammals. These key marker FA may link milk product quality to potential health effects. Forage feeding



increases C18:1  $\omega$ 11 relative to C18:1  $\omega$ 10.<sup>14,55</sup> There is evidence that industrial TFA increase the risk of coronary heart diseases.<sup>56</sup> High levels of single TFA such as C18:1  $\omega$ 9 and C18:1  $\omega$ 10 are considered to be detrimental, whereas ruminant TFA, especially C18:1  $\omega$ 11, are not.<sup>57–59</sup> C18:1  $\omega$ 11 is even regarded positively, mainly owing to its action as CLA c9, $\omega$ 11 precursor.<sup>60</sup> In humans, C18:1  $\omega$ 11 can be transformed into CLA,<sup>18</sup> and this is also the case for ruminants.<sup>61</sup>

#### Differences in AO in milk due to system feeding management

Differences in AO concentrations as a further aspect of milk quality were also associated with higher grass intake. The high levels of  $\beta$ -carotene in B systems and especially in group BLI could be explained by the high concentrations of carotenoids in young grass but also in green cobs, while the concentrations in grass silage and hay are decreased by a factor of 3–4 and ~10 respectively.  $\alpha$ -Tocopherol concentrations are highest in fresh grass, so this AO showed the highest levels in BLI. Havemose *et al.*<sup>62</sup> report higher  $\alpha$ -tocopherol and  $\beta$ -carotene levels in grass silage compared with maize silage and cereals, which explains the low levels of both compounds in CHI, but the low concentration in milk might also be explained by dilution due to higher milk yields in C compared with B systems.<sup>63</sup> Higher  $\beta$ -carotene and  $\alpha$ -tocopherol levels in systems with high amounts of pasture and grass silage are reported.<sup>21</sup> AO in summer milk measured by Butler *et al.*<sup>20</sup> show slightly higher levels for  $\alpha$ -tocopherol and lower levels for  $\beta$ -carotene, but with the same differentiation pattern of higher levels in organic and LI systems as in our study.  $\beta$ -Carotene and  $\alpha$ -tocopherol were determined as predicting variables for 'origin' (Table 4) and were suitable as additional markers of fresh grass intake in the systems' ration.

#### Implications for organic milk production

Currently, authenticity and organic product quality still lack generally agreed and precise definitions,<sup>64,65</sup> not to speak of defined indicators for organic milk or product quality aspects of any other organic product. Consumers' assumptions of higher product quality and higher health value of organic produce are reported.<sup>8</sup> High-quality nutritious food that contributes to preventive health care and wellbeing is a general aim of organic farming, stated in the 'principle of health' by the International Federation of Organic Agriculture Movements.<sup>66</sup> The product quality of organic milk in our study was distinguishable. Desired bioactive substances were present at higher levels in all three lower intensified systems compared with CHI, with highest levels in BLI milk.

The differentiation between conventional and organic milk quality was in accordance with several other European studies, which show higher levels of ALA and/or CLA c9, $\omega$ 11 in organic compared with standard conventional milk<sup>13,20,21,63</sup> and milk products such as cheese,<sup>67,68</sup> butter and cream.<sup>69</sup> However, there might be difficulties in the differentiation of organic milk from specific lower intensified production systems from conventional niche systems such as CLI and other LI systems, as also found by Butler *et al.*,<sup>20</sup> as well as the differentiation of highly intensified organic systems from standard conventional milk.

It was recently shown that the intake of grass-based ruminant products results in substantially higher CLA availability for the consumer than previously estimated.<sup>70</sup> Furthermore, epidemiological studies on the consumption of organic milk of different origins show higher C18:1  $\omega$ 11 and CLA c9, $\omega$ 11 and lower C18:1  $\omega$ 9 and C18:1  $\omega$ 10 concentrations in the breast milk of mothers who consume mainly milk products of organic origin.<sup>71,72</sup> In addition,

the incidence of eczema and allergic sensitization of 2-year-old infants is lower if mothers consume organic dairy products during pregnancy and breastfeeding, which is associated with rumen FA.<sup>12,73</sup> Especially in the group that consumes biodynamic milk products, highest contents of CLA and C18:1  $\omega$ 11 as well as a lower level of C18:1  $\omega$ 9 are found in breast milk.<sup>74</sup>

## CONCLUSION

The examination of complex FA and AO profiles of milk allowed us to differentiate milk from four production systems based on a limited number of markers. Only a few specific FA were necessary to differentiate the system groups, while even fewer were needed to predict the 'intensification level' or the 'origin' of the system. The prediction of 'origin' was mainly based on n-3 PUFA as markers, while the prediction of 'intensification level' was based upon CLA c9, $\omega$ 11 and C18:1  $\omega$ 11. Additionally, CLA  $\omega$ 11,c13 could be suggested as an important marker to differentiate the origin of milk. In accordance with other studies, it was shown that organic summer (outdoor period) milk was consistently different from standard conventional milk in terms of the FA and AO profile.

The feeding of maize silage, concentrates and grass silage in the HI systems decreased total CLA, n-3 PUFA and milk-specific TFA such as C18:1  $\omega$ 11, OBCFA and CLA  $\omega$ 11,c13 levels. The renunciation of a TMR-based ration and the incorporation of fresh grass improved relevant bioactive FA and AO contents in both the organic and conventional systems. Pasture-based organic systems had the potential to produce milk with a nutritionally preferable FA and AO profile. The specific product quality of organic milk, based on higher concentrations of the above-mentioned markers, represented a unique feature. This authentic organic product quality would be threatened if conventional feeding practices and breeding goals with a focus on the highest milk performance per cow were to be implemented in organic dairy production.

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