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Dairy farming system markers: the correlation of forage and milk fatty acid profiles from organic, pasture and conventional systems in the Netherlands

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ABSTRACT

The relationships between the fatty acid (FA) composition in forage and milk (F&M) from different dairy systems were investigated. Eighty milk samples and 91 forage samples were collected from 40 farms (19 organic, 11 pasture and 10 conventional) in the Netherlands, during winter and summer. The FA profiles of F&M samples were measured with gas chromatography. The results showed that the F&M of organic farms were significantly differentiated from the F&M of other farms, both in summer and winter. The differences are likely due to the different grazing strategies in summer and different forage composition in winter. The Pearson's correlation results showed the specific relationship between individual FAs in forages and related milk. A PLS-DA model was applied to classify all milks samples, resulting in 87.5% and 83.3% correct classifications of training set and validation set.

Keywords: Classification; Correlation analysis; Fatty acids; Forage; Milk; Organic

1. Introduction

Nowadays, there is much interest in organic milk, considering its association with environmental, social and economic sustainability (Altieri, 2018). The differences of organic-labelled milk and conventional-labelled milk are due to different farming practices. According to the European Commission regulation (EC) No 889/2008: organic cows should graze in organic grassland without limitation; the organic grassland that is provided to cows should not be treated with pesticides and synthetic fertilizers; and roughage should comprise the largest portion of the cow's daily feed intake. Consumers are willing to pay more for these dairy products. In the Netherlands, there is a type of milk called *weidemelk*, which is pasture milk in English. The farmers that produce this type of milk should allow their cows to graze on outdoor pastures for at least 120 days per year for at least 6 hours per day, but the use of pesticide and synthetic fertilizers are allowed (Liu, Koot, Hettinga, et al., 2018). Dairy companies pay a premium price to the farmers producing pasture milk. However, the price gap between different types of milk makes organic and pasture milk vulnerable to fraudulent practices. To guarantee that the milk is produced according to the regulations, and to protect the rights of stakeholders that obey the regulations, the authenticity of milk should be confirmed.

Recently, various studies have been carried out to determine the differences between organic milk and other types of milk. These studies employed the use of e.g. stable isotope ratios (Chung, Park, Yoon, et al., 2014; Molkentin, 2013), organic volatile compounds (VOCs) (Ueda, Asakuma, Miyaji, et al., 2015; Vasta, D'Alessandro, Priolo, et al., 2012; Villeneuve, Lebeuf, Gervais, et al., 2013), and mineral elements and vitamins (Ellis, Monteiro, Innocent, et al., 2007; Mogensen, Kristensen, Søegaard, et al., 2012; Rey-Crespo, Miranda, & López-Alonso, 2013). In addition to these studies, the fatty acid (FA) profiles of organic and other types of milk, especially focusing on the *n*-3 and *n*-6 family FAs (van Valenberg, Hettinga, Dijkstra, et al., 2013), have also been investigated (Butler, Nielsen, Slots, et al., 2008; Capuano, van der Veer, Boerrigter-Eenling, et al., 2014; Stergiadis, Leifert, Seal, et al., 2012(Stergiadis, Leifert, Seal, et al., 2012)

However, most of the above-mentioned studies just focused on the FA profiles of milk. Research regarding organic feedstuffs and the link between FA profiles of feedstuffs and milks are limited. Hence, it is of value to explore the specific FA profiles in different feedstuffs and to determine if the features in milks and feedstuffs are significantly correlated, especially in uncontrolled conditions as occurs in real life.

Meanwhile, other researchers have focused on the FA metabolism of cows provided with different diets (Adler, Jensen, Thuen, et al., 2013; Leiber, Kreuzer, Nigg, et al., 2005; Willems, Kreuzer, & Leiber, 2014). The ruminal biohydrogenation and apparent transfer rate of long chain unsaturated FA were shown to be partly dependent on the forages (Buccioni, Decandia, Minieri, et al., 2012; Villalba, Provenza, K Clemensen, et al., 2011). Compared with pasture farms (PFs) and conventional farms (CFs), organic farms (OFs) provide different diet profiles to dairy cattle, in terms of forage types and forage ration (Capuano, van der Veer, Boerrigter-Eenling, et al., 2014). Therefore, it would be useful to investigate the correlations between milk FAs and diet FAs in these different dairy production systems. In the Netherlands, the feedstuffs used in dairy farming systems typically consist of forages and concentrates. Forages are produced by the local dairy farms while concentrates are provided by commercial animal feed companies. Farm-produced forages can be divided into fresh forages (herbage) and conserved forages (silage or hay). These forages reflect the features or characteristics of specific farms. The diversity of forages from different farms is relatively high, due to the different types of botany present on different farms, the variation in ratio and quality of raw materials used to make forages, the effect of season, etc. In the Netherlands, the compositions of the silages from OFs, PFs and CFs are different. Although maize is allowed to be added into organic dairy diets, it is not common that organic dairy farmers provide organic maize to cows. Since the use of pesticides in organic farming is forbidden, the production of organic maize is much lower than conventional maize, leading to a higher price of organic maize. Meanwhile, Dutch organic dairy farmers believe that feeding cows with grass is more natural then feeding them with maize. Due to these economic and ecological reasons, few OFs in the Netherlands used organic maize to produce silage. On the other hand, non-organic farmers use more maize in their cows' diets, since it is known to increase the yields of fat and protein in the milk (Elgersma, Ellen, Van der Horst, et al., 2004), which is directly associated with the milk payments in the Netherlands. Furthermore, OFs provide highest ratio of fresh forages to cows in summer. On the contrary, CFs don't provide any fresh forages to cows both in winter and summer.

Since the difference in forages between dairy production systems may be reflected in milk composition (Martin, Verdier-Metz, Buchin, et al., 2005)., the aim of this study was to determine the relationship between the FA profiles of forage and milk from three different dairy production systems (i.e. organic, pasture and conventional) in the Netherlands, and discriminate different milks according

to their FA concentrations Since large differences between seasons is expected, samples were collected both in the winter and summer period.

2. Materials and methods

2.1. Sample collection

During the European summer and winter period, forage and milk samples were collected from 40 dairy farms in the Netherlands (Table 1). The farms were evenly distributed across the country. The winter sample set was obtained from December 2016 to February 2017 and the summer sample set was obtained from July 2017 to August 2017. In the winter, cows from all the systems stayed indoors and were provided with silage. It is because grassland is more fragile in winter and easily damaged if it was over-grazed. In the summer, only organic cows fully grazed because of the low cow density in organic farms, whereas on the PFs, grazing was supplemented by other forages. Cows of the CFs were only provided with silages without access to pasture grazing in the summer. The details of the diets and grazing time for the cows of the different farming systems are shown in Table 1. As there are several factors that may affect the fatty acid profile of herbage during the season (M. Coppa, Farruggia, Ravaglia, et al., 2015; Elgersma, 2015; Revello-Chion, Tabacco, Peiretti, et al., 2011), care was taken to select a time window that is most likely to provide relatively stable samples during the sampling stage. As a result, 80 milk samples and 91 forage samples were collected (Table 1). Milk produced from three subsequent days were temporarily stored in bulk cooling tanks (3°C) before sampling. Prior to sampling, the milk had been stirred for 10 seconds to ensure that a homogenous sample could be taken. From each farm, a 200 ml milk sample was collected in summer and winter, respectively. Fresh herbages (i.e. fresh forages) were cut at normal harvest heights at six locations within the grazing area of the organic and pasture grasslands. Information on the botanical compositions of the sward was obtained from the farmers. Instead of the bulk silage storage facilities silage samples (i.e. conserved forages) were collected from the stalls directly (500 g from each farm), when they were provided to the cows. The ration composition was reported by the dairy farmers. All the forage samples were stored in tightly sealed plastic bags, to exclude air. The milk and forage samples were stored at −18°C until analysis.

2.2. Forage fat extraction

A 5.0 g sample of forage was weighed (to the nearest 0.01 g) into a 100 ml conical flask. The sample was then mixed with 4 g of sodium sulphate, after which 25 ml of a chloroform:methanol mixture

(2:1, v/v) was added and stirred for 20 min with a magnetic stirrer. The solution was then filtered into a clean 100 ml conical flask. The extraction was repeated two more times with the chloroform:methanol mixture and the filtrate collected in the same flask. The final filtrate was dried under a stream of nitrogen gas (with a maximum temperature of 45°C).

2.3. Milk fat extraction

Milk samples (5 ml) were defrosted in a refrigerator overnight at 4°C. The liquid samples were centrifuged for 10 min at 2000 g (Avanti J-25, Beckman Coulter Inc, IN, USA). The resulting top cream layer was collected by spoon and heated in a water bath (Precision GP 20, Thermo Fisher Scientific, MA, USA) for 10 min at 38°C, followed by a 10 min sonication in a ultrasonic bath (Ultrasoon Reiniger 13 L, HBM Machines B.V., Netherlands). The final milk fat extract was obtained after another five-minute centrifugation (1600 g).

2.4. Fatty acid methyl esters (FAME) analysis

The fat extracts of the respective forage and milk samples were analysed with a gas chromatograph instrument, GC16958 (Agilent 7890A, Agilent Technologies, Palo Alto, CA, USA), according to NEN-ISO 1740:2004 | IDF 6. The GC was equipped with a 100 m × 0.25 mm × 0.2 µm film thickness fused silica capillary column (Varian, Palo Alto, CA) coupled to a flame ionization detector (temperature: 275°C). Approximately 70 mg of fat was weighted in a 30 ml sterile, screw top plastic bottle, mixed with 5 ml of the internal standard solution: 500 mg of C13:0 triglyceride and 500 mg of C11:0 FAME in 250 ml tert-butyl methyl ether. For the transesterification step, 5 ml of a methanol sodium methoxide solution (5%, m/v) was added, followed with the addition of 2 ml hexane and 10 ml neutralization solution after 180 s and 210 s, respectively. The mixture was then vortexed for 30 s and centrifuged for 5 min, after which 1 ml of the supernatant was removed with a pipette and transferred to a 1.5 ml GC amber glass vial with a magnetic crimp cap. Each sample was weighed and measured in duplicate. The concentrations of FAs were expressed as mg/100 mg total fat. Average values of the duplicates were used for data analysis. All samples were analysed in duplicate. All the chemicals that were used were ACS grade, and purchased from Sigma Aldrich (St. Louis, MO, USA). All samples were analysed in duplicate.

2.5. Data analysis

According to the results of the Shapiro-Wilk test, the absolute FA concentrations, and log transferred scores, were not normally distributed, which was due to the large variance among farms. The Kruskal-Wallis test was therefore applied to determine if there were any significant differences, at a significance level of 5% (P=0.05), between different types of forages (Liu, Parra, et al., 2018). Pairwise comparisons were carried out by the Mann-Whitney U-test to investigate differences in FA levels. In a similar manner, the FA compositions of the milk samples were also analysed using the same tests. To control the false discovery rate (FDR), *P* values were adjusted using Benjamini-Hochberg (BH) adjustment (Benjamini & Hochberg, 1995). Principal component analysis (PCA) was used to visualise relations between samples. To balance the weights of different variables, raw data were auto-scaled before PCA. The correlations between FA profiles of milk and forages were evaluated by calculating Pearson's correlation coefficients (r) (Snedecor & Cochran, 1980). After the correlation analysis, partial least squares discrimination analysis (PLS-DA) algorithm was applied to build a classification model with 70% of the milk samples (training set) to discriminate milks from different farming system (organic, pasture and conventional). The rest 30% of the milk samples (validation set) was used to validate the model. The performance of the model was evaluated by the overall accuracy. The best pre-processing methods and components number were determined by the results of leave-five-out cross validation. All the statistical analyses were conducted using R 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Fatty acid profiles of forages from the three different production systems

The FA profile of each forage type is shown in Table 2. The dairy production system had a strong effect on the forage FA composition. Only C18:1TFA, C18:4*n*3 and C20:2*n*6 did not significantly differ between the production systems (P>0.05). According to the pairwise comparisons, the FA profiles between fresh forages (herbage) and conserved forages (silage) were significantly different. Compared with summer organic herbages, summer conventional silages and winter silages had lower percentages of C18:3*n*3 and higher percentages of C18:2*n*6 and C16:0. In fresh forages (summer organic herbages and summer pasture herbages), the dominant FAs were linolenic acid (C18:3*n*3), linoleic acid (C18:2*n*6) and palmitic acid (C16:0). The summer organic herbages had higher concentrations of C16:0 and

C18:2*n*6, and a lower concentration of C18:3*n*3 than the summer pasture herbages. The significant differences between organic and non-organic conserved forages were observed in the proportions of C18:1*n*9, C18:2*n*6, C18:3*n*3 and monounsaturated fatty acids (MUFA) (Table 2). To obtain a better view on the differences between different sample groups, FA compositions of different forages were subjected to PCA and the plots resulting from this are shown in Fig. 1. In the summer forage PCA bi-plot (Fig. 1a), fresh forages are clearly separated from conserved forages. The dominant FAs that are closely related to fresh forages were C18:3*n*3 and PUFA, while MUFA, C18:1*n*9 and C18:2*n*6 are more associated with conserved forages. Regarding the conserved forages in winter (Fig. 1b), organic silage samples are grouped in the middle part of the plot, while conventional silage samples are grouped in the right hand side of the plot. When combining summer and winter samples (Fig. 1c), the winter and summer pasture silages are grouped with the conventional silage samples and with winter organic silage, but share relatively more overlapping area with winter pasture silage and summer conventional silage along PC1 (Fig. 1c). The FA C18:1*n*9 was observed to be an indicator of winter conventional silage in Fig. 1b, matching with the result from Table 2, which shows a significant higher level of C18:1*n*9 in winter conventional silage compared to winter organic silage. Compared to Fig. 1a, fewer major FAs show clear relations with certain forage groups in Fig. 1b, similar to the result from Table 2.

3.2. The fatty acid profiles of milk from the three production systems

Table 3 lists the FA profiles of the different milk samples collected in the summer and winter periods. Regarding the predominant FAs, summer organic milk had a significantly lower concentration of C16:0 than other types of milk. The concentrations of C16:0 in winter milk (including organic, pasture and conventional) were stable, and were also similar to the concentrations of C16:0 in summer conventional milk. Another abundant FA in the milk was C18:1*n*9, ranging from ~18% to ~21% (Table 3). The proportions of C18:1*n*9 in summer organic milk was the highest, followed by summer pasture milk, while the concentrations in other types of milk were the lowest (Table 3). Table 3 also shows that the concentrations of conjugated linoleic acid (CLA) was significantly higher in summer organic milk compared to the other types of milk. Total saturated and polyunsaturated fatty acids (SFA and PUFA, respectively) were more stable between summer and winter in the pasture and conventional milk compared to the organic milk. An overview of the differences between different types of milk and related FAs is shown in Fig. 2 by PCA. The overlapping area of summer milk (Fig. 2a) is larger than the overlapping area of winter milk (Fig. 2b), which suggests that there were greater differences between

organic, pasture and conventional milk in the winter. In fact, the organic milk was more separated from the conventional milk in winter than in summer, as shown in Fig. 2a&b. In Fig. 2a, C18:1*n*9, PUFA, C18:3*n*3 and CLA all strongly associated with summer organic milk, while C18:2*n*6, PUFA, C18:3*n*3 and CLA associated with winter organic milk in Fig. 2b. With the majority of differentiating FAs being the same in summer and winter, there is actually quite a good overlap between summer samples and winter samples, even though they are not identical. Overall the milk FA profiles shown a clear difference between both production system and season.

3.3. Correlation between the fatty acid profiles of forages and milk of the three production systems

To link the FAs from forage to milk, the relationships between the FAs in the milk and the dominant FAs in forages, including six individual FAs (C16:0, C16:1*n*7, C18:0, C18:1*n*9, C18:2*n*6 and C18:3*n*3) and four FA groups (CLA, SFA, MUFA and PUFA) were evaluated with Pearson correlation coefficients. Fig. 3 shows the significant (P<0.05) Pearson correlation coefficients between different types of forages and milk. It is interesting to note that in winter, C18:1*n*9 in organic milk (i.e. cows consuming winter organic silage) and pasture milk (i.e. cows consuming winter pasture silage) showed significant positive correlation with stearic acid (C18:0) in the forages (Fig 3). The proportions of PUFA in milk were observed to have a significant positive correlation with concentration of PUFA and C18:1*n*9 in the winter organic (r=0.49; P=0.03 and r=0.51; P=0.04 respectively) and pasture forages (r=0.62; p=0.04 and r=0.63; p=0.03 respectively). According to the Pearson correlations (Fig. 3), the amount of CLA was linked to the proportions of C18:3*n*3 in summer organic forages, winter organic forages and winter pasture forages (r=0.51; p=0.02, r=0.55; p=0.03 and r=0.72; p=0.01, respectively).

3.4. Classification model

To explore the discriminant capability of GC analysis in classifying different milks, a classification model was developed based on a supervised pattern recognition algorithm, PLS-DA. According to the results of cross validation, autoscale was selected as the pre-processing method and the number of the components was set as five. Under these circumstances, the overall accuracies of the training set and validation set were 87.5% and 83.5% respectively (Supplementary 1). In terms of the results of the prediction dataset, 15.4% of the organic milk samples (2/13) were classified as pasture milk and 7.2% of the organic milk samples (1/15) was classified as conventional milk; 16.7% of the conventional milk samples (1/6) was classified as pasture milk; all the pasture milk samples were correctly classified as

pasture milk. The details of the confusion matrix of the prediction set were presented in (Supplementary 2).

4. Discussion

4.1. Effect of production systems on forage FA profiles

Compared with the silages from CFs, silages from OFs had lower concentrations of C18:1*n*9, C18:2*n*6 and MUFA, and higher concentrations of C18:3*n*3. It was due to the different percentages of maize silages in organic forages and conventional forages. Higher levels of maize silages tend to increase the percentages of C18:1*n*9 and C18:2*n*6, and decrease the percentages of C18:3*n*3 (Keady, Kilpatrick, Mayne, et al., 2008). Meanwhile, the use of maize silage could also lead to the differences in milk MUFA level, since they reflect the differences in C18:1*n*9, which is the most dominate MUFA in silage forages.

Another characteristic in Dutch organic dairy farms is the distinct botanical species growing in the fields (Table 1). Floral species vulnerable to herbicides and intensive management, such as *Fabaceae, Brassicaceae* and *Polygonaceae*, were previously shown to be more prominent in the fields of OFs. This kind of biodiversity is the result of banned usage of herbicides in organically cultivated fields (Hyvönen, Ketoja, Salonen, et al., 2003). Furthermore, organic farmers cultivate clover herbage for N_2 fixation as an alternative method for nitrogen introduction (Elgersma & Hassink, 1997), while CFs apply synthetic nitrogen fertilizers for that purpose. Clover herbage contains relatively more C16:0 and less C18:3*n*3, compared to grass herbage (Vanhatalo, Kuoppala, Toivonen, et al., 2007), similar to the findings of this study (Table 2), explaining the differences of C16:0 and C18:3*n*3 between summer organic herbages and summer pasture herbages.

Furthermore, the differences of C18:3*n*3 between summer organic herbages and summer pasture herbages could be explained by the utilizing of synthetic fertilizer as well. Since pasture farmers are allowed to apply synthetic fertilizers, it is easier for them to increase the nitrogen content in the field, which could significantly ($P<0.01$) increase the concentrations C18:3*n*3 in timothy grass (Boufaïed, Chouinard, Tremblay, et al., 2003).

In contrast to the previous study (Keady, et al., 2008), significant differences in C16:0 were not observed between different silages (Table 2). This could be due to the large diversity within pasture and conventional silages, which covered the variances of silages from different systems, and made the

differences between groups less significant. Since the current study was carried out in natural uncontrolled conditions, the grass: maize silage ratio used per farm was inconsistent. Silages from different farms were made in different ways using different ratios of grass and maize. These factors could generate differences among different farms and forages (Mohd-Setapar, Abd-Talib, & Aziz, 2012), and may thus be responsible for the large differences in feed FA compositions (Table 2).

From Fig. 1c, it also appears that, despite the effect of different harvesting seasons and cutting times, which are known to have a significantly impact on the quality of silage (Elgersma, et al., 2003; Kuoppala, Rinne, Nousiainen, et al., 2008), FA differences between conventional silage in winter and summer are negligible. The latter was also applicable to winter and summer pasture silage, showing negligible differences in FA concentrations in Table 2. One of the potential explanation was that dairy farmers mix the silages harvested in both harvesting season to make the ensiled forages quality more constant along the year. The forage analysis thus shows that production system and season, but especially the forage processing procedure, leads to differences in FA profiles.

4.2. Effect of production systems on milk FA profiles

The differences of C16:0 in summer organic, pasture and conventional milks could be explained by the different amount of silages in the diets. Previous studies (Capuano, Gravink, Boerrigter-Eenling, et al., 2015; Capuano, et al., 2014; Coppa, Ferlay, Chassaing, et al., 2013) reported considerably more C16:0 in milk derived from silage-fed cows. In summer time, only cows from OFs were fed with pure fresh herbage, without silage, while cows from PFs were fed with fresh herbage mixed with silage and conventional cows were only fed silage. In the same way, since all cows were fed with silage during winter, the concentrations of C16:0 did not show significant differences between different winter milk types. Similarly, high proportions of C18:1*n*9 in summer organic and pasture milk (Table 3) may be explained by cows grazing on fresh herbage, which is in accordance with the research carried out by Capuano et al. (2014).

Even though PF cows were partly grazing in the summer time, the differences of SFA and PUFA in pasture milk between winter and summer were small (Table 3). In the previous study mentioned, Capuano, et al. (2015) listed several potential factors that would obscure the unique characteristics of milk derived from grazing cows: grazing time in PFs may be insufficient; a mixture of milk from different farms may submerge some characteristics; fresh cut herbage is provided to indoor cows. However, in our study, milk samples from different farms were analysed separately and conventional cows were

287 indoors throughout the year without fresh herbage intake. The most likely reason leading to the similar
288 results is the unlimited supply of silage in PFs, even during summer time.

289 *4.3. Effect of production systems on forage-milk FA correlations*

290 According to the number of significant correlations (Fig. 3), long chain milk FAs (with more than
291 16 carbon atoms) possessed a comparable stronger relationship with these critical forage FAs. This
292 result is likely related to the FA uptake and secretion pathways. FA uptake pertains to dietary long-chain
293 FAs (Doreau, Meynadier, Fievez, et al., 2016), which are partly biohydrogenated and desaturated in
294 the rumen and mammary gland, respectively (Elgersma, et al., 2004). In the mammary gland, short and
295 medium chain FAs are *de novo* synthesised, while long chain FAs are absorbed from the blood and
296 originate from both feed and fat deposits. Altogether, these processes cause a more direct relation
297 between forage and milk composition for long chain FAs, compared to short and medium chain FAs.
298 Stearic acid (C18:0), from feed or formed during biohydrogenation, can be absorbed in the digestive
299 tract and transferred to the mammary gland, where it can be desaturated to oleic acid (C18:1*n*9),
300 explaining the highlighted correlations between C18:0 and C18:*1n9* in winter organic and PFs (Fig. 3) .
301 Meanwhile, Doreau, et al. (2016) reported a linear relationship between the amount of absorbable PUFA
302 in the small intestine and the amount of PUFA intake from feedstuffs. Since the biohydrogenation
303 specifically occurs in the rumen, after the absorption in the small intestine, the absorbed PUFAs could
304 end up in the milk, illustrating the correlations between forage PUFA and milk PUFA in winter organic
305 and PFs.

306 Due to the perceived public health effect, another group of FAs, which draws consumers'
307 attention, is CLA. As the most abundant FA in the diet, C18:3*n*3 is partly biohydrogenated in the rumen
308 by the rumen bacterium, *Butyrivibrio fibrisolvens*. Increasing intakes of C18:3*n*3 by cows linearly
309 increases milk CLA output (Elgersma, Ellen, Dekker, et al., 2003). Meanwhile, the activity of *B.*
310 *fibrisolvens* is highly dependent on rumen pH and the microbial biohydrogenation of C18:3*n*3 will be
311 reduced if the rumen pH decreases (Pariza, Park, & Cook, 2000), which may be a result of the
312 consumption of maize and concentrate-based diets (Bessa, Santos-Silva, Ribeiro, et al., 2000; Doreau,
313 et al., 2016). In the current study, significantly higher proportions of CLA in organic milk were observed
314 both in summer and winter. However, the reasons leading to such significant abundance were different.
315 During the summer period, the higher percentages of CLA was due to grazing, while during the winter
316 period, it resulted from the silage composition on OFs, which contained no or less maize than

conventional silage. Thus, the management and forages compositions and types are responsible for the distinct percentages of CLA in organic milk in summer and winter, respectively.

In terms of different production systems, the differences between forage-milk relationships in summer OFs and summer PFs could be mainly related to higher biodiversity levels in OFs. In the OFs, the percentages of grass in whole plant cover were lower than the percentages in the PFs (Table 1). Herbs contain higher levels of plant secondary compounds, such as polyphenol, than grass (Thorpe, Archer, & DeLuca, 2006; Willems, et al., 2014). It has been shown previously that the level of total extractable phenols may have an impact on the process of lipolysis and biohydrogenation (Gerson, John, & King, 1986). Higher levels of phenolic compounds were shown to reduce the biohydrogenation of C18:3*n*3 (Jayanegara, Kreuzer, Wina, et al., 2011). Besides phenols, fibre also showed different levels between grass, legume and herb (Willems, et al., 2014). A high level of fibre may inhibit the process of biohydrogenation and can thereby result in different relationships between forage FAs and milk FAs. Moreover, polyphenol oxidase released from some legume forages can increase lipid protection from endogenous lipolytic activity (Buccioni, et al., 2012). For example, adding red clover, which contains higher levels of polyphenol oxidase than grass, into diet, increased the transfer rate of C18:3*n*3 and C18:2*n* from forages to milk. A part of the major forage fatty acids, like C18:3*n3*, C18:2*n6* and C18:1*n9* could pass the rumen without being biohydrogenated. This can occur on pasture when cows graze grass and herbs in a young development stage, which then gives high forage intake and high passage rate of forage particles through the rumen. Moreover, Willems, et al. (2014) pointed out that the selective eating behaviour of grazing animals differs between farms with different levels of biodiversity. Ruminants tend to select plants to avoid negative impacts of single substances, which could theoretically effect the rumen passage rate (Villalba, et al., 2011). In addition to the different levels of biodiversity, the different relationships in OFs and PFs could be due to different FA levels in the organic forages and pasture forages as well. In our study, correlations between the proportions of milk PUFA and forage PUFA in winter PFs ($r=0.62$) were stronger than the correlations in winter OFs ($r=0.49$). On the contrary, PUFA portions in winter organic forages were higher than the portions of PUFA in winter pasture forages. According to the meta-analysis conducted by (Khiaosa-ard, Kreuzer, & Leiber, 2015), the apparent recovery of PUFA is higher at low dietary PUFA levels, and decreases when the dietary PUFA levels increase.

According to Fig. 3, the number of significant correlations was lowest in conventional dairy system (both in winter and summer). The less number of significant correlations could be due to higher percentages of concentrates provided conventional dairy systems (Table 1). Butler, et al (2008) reported that cereal-based concentrates could reduce the CLA content in milk. Such conclusion matches the results in the current research that the present of the significant correlations between milk CLA and forage FAS were only observed in organic and conventional dairy systems. Coppa, et al (2013) evaluated the relationships between milk FAs and farming practise as well and develop FA compositon prediction model based farming practices. According to their results, in the fresh herbage-derived milk samples, the intake of concenetrates has an impact on the C18 unsaturated FAs except C18:3n3; while in the conserved forage-derived milk samples, the intake of concenetrates has no relationship with the concentrations of C14:0, C16:0, C181n9, C18:2n6 and C18:3n3. The concentrations of these FAs, which are not significantly affected by the concentrates intake, were found to own significant correlation(s) with the concentrations of different feed FAs (Fig 3). Furthermore, Coppa, et al (2013) suggested that the relationship between concetrates intake and milk FAs differ between fresh-based diet and conserved based-diet. It could lead to different correaltion patterns in the current research (Fig. 3).

4.4. Classification model evaluation

The pilot pattern recognition model was developed based on the concentrations FAs in different milks. The overall accuracy suggested that combined with chemometrics, different milks from different seasons could be correctly classified (20/24) according to their production systems. According to the results presented in the Supplementary 2, 2/3 of the misclassified organic milk samples were classified as pasture milk samples while the misclassified conventional sample was classified as pasture sample. Such kind of misclassifications matches with the previous conclusion (Capuano, et al, 2014) that pasture milk is a kind of intermediate milk between organic and conventional milk. In terms of the model contributions of different variables, the two FAs that had highest model projection scores were CLA (1.42) and C18:3n3 (1.31). The model projection score illustrates the role of each variable in terms of bulding classification models (Capuano, et al, 2014). Variables with higher scores stand for more contribution in classifying samples precisely. Hence, these three FAs played important roles in discriminat different milks, which are in accordance with findings in the previous sessions. Furthermore,

since the model was developed and validated with milks from winter and summer, it proves that it is possible to discriminate different milks from different seasons with one model.

5. Conclusions

The current study investigated if there are significant differences between forages and milk from different production systems, as well as if there is any unique relationship between milk and the related forage per system, under uncontrolled conditions. It is important because those are the products that consumers buy in daily life. The study provided the differences between the FA profiles of related forages and a detailed assessment of the relations between milk and forage in three different dairy farming systems. During the winter period, the forages from CFs were poorer in C18:3*n*3 but richer in C18:2*n*6 and MUFA than the forages from OFs. During the summer period, the forages from PFs had higher concentrations of C18:3*n*3 and lower concentrations of C16:0 and C18:2*n*6 than the forages from OFs. The variation of the conventional between seasons (summer and winter) was small as the diets were similar for both seasons, minimising the seasonal effect. For organic and pasture the seasonal effect was greater as there is a distinct change in feeding practices for summer and winter. The variation in FA concentration between systems was due to the different feeding practises and resulting diets of the animals. The study revealed that higher percentages of CLA in summer organic milk were due to grazing management in summer OFs, while higher percentages of CLA in winter organic milk were due to less maize in silage in winter organic forage.

Overall, the results show that the organic cow's diet is different from those in other systems, which is particularly due to the grazing management in summer and silage composition in winter, and results in distinct features of the organic milk FA profile, which could be used to distinguish milks from different production systems.

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537

538 **Table 1**

539 List of forages, types of milk and abbreviations used.

Farm	Cattle breed	Season*	Type of milk	Forages**				Concentrate levels (% of DMI)	Grazing time
				Type of forage	Forage ingredients and composition (% of DMI)	Dry matter (DM, g/100g)	Crude protein (g/100g DMI)		
Organic	Holstein, Red Holstein, Holstein Friesian, Montbéliarde, Brown Swiss, Jersey	Summer	Summer organic milk (SOM, n=19)	Summer organic herbage (n=19)	fresh grass: 63±3; fresh legumes (including red clover, white clover and alfalfa): 18±3	185±33	22±2	18±5	>12 hrs
		Winter	Winter organic milk (WOM, n=19)	Winter organic silage (n=19)	Grass silages: 70±6	374±97	15±3	22±7	0 hr
Pasture	Holstein, Red Holstein, Holstein Friesian, Brown Swiss, Jersey	Summer	Summer pasture milk (SPM, n=11)	Summer pasture herbage (n=11)	fresh grass: 40±3	207±21	21±2	25±6	4-8 hrs
				Summer pasture silage (n=11)	grass silage: 20±4 maize silage: 15±4	390±56	16±1		
		Winter	Winter pasture milk (WPM, n=11)	Winter pasture silage (n=11):	grass silage: 40±5 maize silage: 35±3	419±55	15±4	24±8	0 hr
Conventional	Holstein, Red Holstein, Holstein Friesian, Meuse-Rhine-Yssel, *Blaarkoppen*	Summer	Summer conventional milk (SCM, n=10)	Summer conventional silage (n=10)	grass silage: 39±3 maize silage: 34±5	354±59	13±2	26±7	0 hr
		Winter	Winter conventional milk (WCM, n=10)	Winter conventional silage (n=10)	grass silage: 40±3 maize silage: 33±5	362±49	14±3	25±7	0 hr

540 *: Number of samples

**: The values of dry matter and crude protein are the mean values of 8 reprensentative samples from each group

Table 2

Fatty acid compositions (%) in summer organic herbage (SOH), summer pasture herbage (SPH), summer pasture silage (SPS), summer conventional silage (SCS), winter organic silage (WOS), winter pasture silage (WPS) and winter conventional silage (WCS): mean concentrations ± standard deviations and statistical relevance of differences between forages.*

Fatty acids	Summer				Winter			*P*
	SOH (n=19)	SPH (n=11)	SPS (n=11)	SCS (n=10)	WOS (n=19)	WPS (n=11)	WCS (n=10)	
C6:0	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.1^{ab} ± 0.1	0.1^{ab} ± 0.1	0.2^{b} ± 0.2	0.1^{ab} ± 0.1	0.1^{ab} ± 0.1	<0.01
C10:0	0.1^{a} ± 0.0	0.1^{a} ± 0.0	0.1^{a} ± 0.1	0.2^{a} ± 0.2	0.2^{a} ± 0.1	0.1^{a} ± 0.1	0.2^{a} ± 0.2	<0.05
C12:0	0.2^{a} ± 0.1	0.2^{ab} ± 0.0	0.4^{b} ± 0.2	1.4^{c} ± 2.2	0.4^{bc} ± 0.2	0.4^{b} ± 0.2	1.4^{abc} ± 2.2	<0.01
C14:0	0.4^{a} ± 0.1	0.3^{a} ± 0.1	0.5^{ab} ± 0.2	0.7^{ab} ± 0.7	0.7^{b} ± 0.2	0.5^{ab} ± 0.2	0.7^{ab} ± 0.7	<0.01
C15:0	0.2^{a} ± 0.0	0.1^{a} ± 0.0	0.2^{b} ± 0.1	0.1^{a} ± 0.1	0.4^{b} ± 0.2	0.2^{a} ± 0.1	0.1^{a} ± 0.1	<0.01
C16:0	17.1^{b} ± 1.1	15.3^{a} ± 1.0	13.5^{a} ± 2.0	12.7^{a} ± 3.0	14.0^{a} ± 1.5	13.2^{a} ± 2.2	13.0^{a} ± 3.1	<0.01
C16:1*n*7	3.2^{c} ± 0.2	3.0^{c} ± 0.3	1.5^{ab} ± 0.6	0.9^{a} ± 0.3	2.0^{b} ± 0.2	1.6^{ab} ± 0.5	1.0^{a} ± 0.4	<0.01
C17:0	0.2^{b} ± 0.0	0.2^{ab} ± 0.01	0.2^{b} ± 0.0	0.1^{ab} ± 0.1	0.2^{ab} ± 0.1	0.2^{ab} ± 0.0	0.1^{a} ± 0.0	<0.01
C18:0	2.0^{b} ± 0.2	1.5^{a} ± 0.2	1.8^{ab} ± 0.5	1.9^{ab} ± 0.3	2.0^{ab} ± 0.5	1.8^{ab} ± 0.4	2.0^{ab} ± 0.9	<0.01
C18:1TFA	0.0^{a} ± 0.0	0.1^{a} ± 0.1	0.2^{a} ± 0.2	0.09^{a} ± 0.1	0.32^{a} ±0.1	0.1^{a} ± 0.2	0.1^{a} ± 0.1	
C18:1*n*9	2.7^{a} ± 0.4	2.2^{a} ± 0.6	8.1^{bc} ± 4.77	15.3^{c} ± 6.1	5.7^{b} ± 3.0	10.2^{bc} ± 6.6	13.9^{c} ± 4.6	<0.01
C18:2TFA	0.0^{a} ± 0.0	0.1^{a} ± 0.0	0.1^{a} ± 0.1	0.1^{a} ± 0.2	0.2^{a} ± 0.4	0.1^{a} ± 0.1	0.1^{a} ± 0.1	<0.05
C18:2*n*6	14.1^{b} ± 0.9	12.2^{a} ± 1.2	24.2^{cd} ± 7.1	33.3^{d} ± 9.0	19.4^{c} ± 5.1	25.4^{cd} ± 7.0	33.5^{d} ± 10.4	<0.01
C18:3*n*3	53.1^{c} ± 2.8	58.2^{d} ± 3.8	40.1^{bc} ± 10.8	25.8^{a} ± 11.4	42.6^{b} ± 7.8	36.9^{ab} ± 9.4	26.3^{a} ± 11.8	<0.01
C18:3TFA	0.3^{b} ± 0.1	0.3^{b} ± 0.0	0.2^{ab} ± 0.1	0.1^{a} ± 0.1	0.3^{ab} ± 0.2	0.3^{ab} ± 0.1	0.2^{a} ± 0.1	<0.01
C18:4*n*3	0.1^{a} ± 0.1	0.1^{a} ± 0.1	0.1^{a} ± 0.1	0.1^{a} ± 0.2	0.2^{a} ± 0.2	0.1^{a} ± 0.1	0.1^{a} ± 0.2	
C19:0	0.3^{b} ± 0.1	0.2^{a} ± 0.1	0.2^{ab} ± 0.3	0.3^{ab} ± 0.3	0.2^{ab} ± 0.2	0.2^{ab} ± 0.3	0.3^{ab} ± 0.2	<0.05
C20:0	0.3^{b} ± 0.1	0.2^{a} ± 0.1	0.4^{bc} ± 0.2	0.4^{cd} ± 0.1	0.5^{abc} ± 0.3	0.4^{c} ± 0.1	0.4^{bc} ± 0.1	<0.01
C20:2*n*6	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.0^{a} ± 0.0a	0.0^{a} ± 0.0	0.0^{a} ± 0.0	0.0^{a} ± 0.0	
C20:*3n3*	0.1^{a} ± 0.1	0.1^{ab} ± 0.2	0.1^{ab} ± 0.2	0.1^{ab} ± 0.1	0.3^{b} ± 0.2	0.2^{ab} ± 0.1	0.1^{ab} ± 0.1	<0.01
C20:4*n*6	0.7^{a} ± 0.2	0.6^{a} ± 0.2	0.2^{a} ± 0.5	0.3^{a} ± 0.3	1.0^{a} ± 0.9	0.7^{a} ± 0.7	0.5^{a} ± 0.4	<0.01
C22:0	0.7^{b} ± 0.1	0.6^{b} ± 0.1	0.6^{ab} ± 0.2	0.4^{a} ± 0.1	0.7^{b} ± 0.3	0.6^{ab} ± 0.2	0.4^{a} ± 0.1	<0.01
C24:0	0.6^{c} ± 0.1	0.4^{b} ± 0.1	0.4^{ab} ± 0.2	0.3^{a} ± 0.1	0.5^{bc} ± 0.12	0.4^{ab} ± 0.1	0.3^{ab} ± 0.1	<0.01
CLA	0.2^{a} ± 0.3	0.1^{a} ± 0.2	2.5^{b} ± 1.0	1.9^{b} ± 1.2	2.8^{b} ± 1.6	2.2^{b} ± 1.1	2.09^{b} ± 1.2	<0.01
SFA	21.9^{b} ± 1.6	18.9^{a} ± 1.5	18.4^{a} ± 2.5	18.5^{ab} ± 3.7	20.1^{ab} ± 2.5	18.2^{a} ± 2.5	19.1^{ab} ± 4.0	<0.01
MUFA	6.0^{ab} ± 0.4	5.6^{a} ± 0.7	9.8^{abc} ± 4.3	16.3^{c} ± 5.6	8.0^{b} ± 3.1	12.0^{bc} ± 6.5	15.0^{c} ± 4.4	<0.01
PUFA	68.5^{b} ± 1.9	71.8^{b} ± 2.7	67.6^{ab} ± 4.1	61.6^{a} ± 5.1	66.9^{ab} ± 4.4	65.8^{ab} ± 5.0	62.8^{a} ± 5.2	<0.01

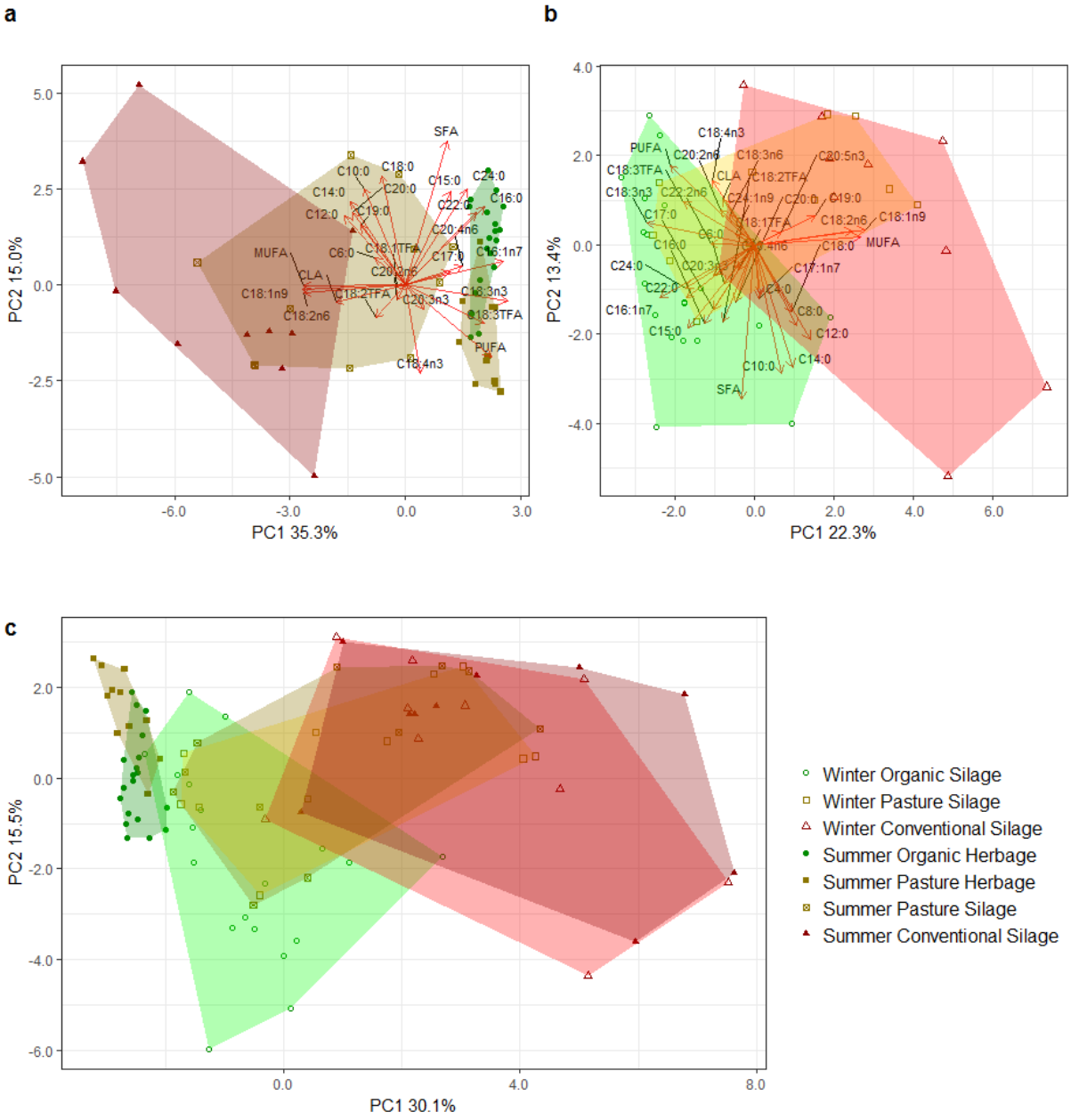
* (TFA) *Trans* fatty acid; (CLA). Conjugated linoleic acids: *cis-9, trans-11* C18:2; (SFA).Saturated fatty acid; (MUFA).Monounsaturated fatty acid (PUFA).Polyunsaturated fatty acid; $^{a\text{-}d}$ Data with different superscript letters are significantly different (P<0.05) according to the Mann-Whitney U-test.

Table 3

The means (± standard deviations) of the fatty acid compositions (%) of summer organic milk (SOM), summer pasture milk (SPM), summer conventional milk (SCM), winter organic milk (WOM), winter pasture milk (WPM) and winter conventional milk (WCM).

Fatty acids	Summer			Winter			P
	SOM (n=19)	SPM (n=11)	SCM (n=10)	WOM (n=19)	WPM (n=11)	WCM (n=10)	
C 4:0	$2.6^{a} \pm 0.2$	$2.5^{a} \pm 0.1$	$2.6^{a} \pm 0.2$	$2.5^{a} \pm 0.2$	$2.3^{a} \pm 0.3$	$2.4^{a} \pm 0.2$	<0.05
C 6:0	$2.0^{a} \pm 0.1$	$2.0^{a} \pm 0.1$	$2.0^{a} \pm 0.1$	$2.0^{a} \pm 0.1$	$2.0^{a} \pm 0.1$	$2.0^{a} \pm 0.1$	
C 8:0	$1.3^{a} \pm 0.1$	$1.2^{a} \pm 0.0$	$1.2^{a} \pm 0.1$	$1.3^{a} \pm 0.1$	$1.2^{a} \pm 0.1$	$1.3^{a} \pm 0.1$	
C10:0	$2.8^{a} \pm 0.3$	$2.9^{a} \pm 0.1$	$2.9^{a} \pm 0.4$	$3.0^{a} \pm 0.3$	$2.9^{a} \pm 0.2$	$3.1^{a} \pm 0.2$	
C12:0	$3.2^{a} \pm 0.4$	$3.9^{b} \pm 0.2$	$3.6^{ab} \pm 0.5$	$3.5^{ab} \pm 0.4$	$3.8^{ab} \pm 0.4$	$3.9^{ab} \pm 0.6$	<0.01
C14:0	$10.7^{a} \pm 0.7$	$11.4^{ab} \pm 0.5$	$10.9^{ab} \pm 0.9$	$11.7^{ab} \pm 0.5$	$11.3^{ab} \pm 0.6$	$11.3^{ab} \pm 0.4$	<0.01
C14:1	$1.0^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.9^{a} \pm 0.4$	$1.1^{a} \pm 0.4$	$1.5^{a} \pm 0.5$	<0.01
C15:0	$1.7^{b} \pm 0.2$	$1.6^{ab} \pm 0.2$	$1.4^{a} \pm 0.2$	$1.5^{ab} \pm 0.4$	$1.8^{b} \pm 0.2$	$1.^{ab} \pm 0.2$	<0.01
C16:0	$26.7^{a} \pm 1.7$	$29.4^{ab} \pm 2.2$	$32.3^{b} \pm 2.7$	$31.6^{b} \pm 2.7$	$31.7^{b} \pm 2.4$	$31.2^{b} \pm 2.3$	<0.01
C16:1*n*7	$3.4^{a} \pm 0.5$	$3.7^{a} \pm 0.7$	$3.9^{a} \pm 0.6$	$3.3^{a} \pm 1.0$	$3.8^{a} \pm 1.1$	$4.1^{a} \pm 1.3$	
C17:0	$0.7^{b} \pm 0.0$	$0.7^{ab} \pm 0.1$	$0.6^{ab} \pm 0.1$	$0.7^{ab} \pm 0.1$	$0.7^{ab} \pm 0.1$	$0.6^{a} \pm 0.0$	<0.01
C17:1*n*7	$0.3^{ab} \pm 0.1$	$0.3^{ab} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.4^{b} \pm 0.1$	$0.3^{ab} \pm 0.1$	$0.3^{ab} \pm 0.1$	<0.01
C18:0	$12.6^{b} \pm 2.0$	$10.8^{ab} \pm 2.0$	$9.8^{a} \pm 1.8$	$10.7^{ab} \pm 1.6$	$9.7^{a} \pm 1.3$	$9.5^{ab} \pm 1.6$	<0.01
C18:1*n*9	$21.4^{c} \pm 1.5$	$20.3^{bc} \pm 1.2$	$19.9^{b} \pm 1.6$	$17.7^{a} \pm 2.0$	$18.2^{ab} \pm 2.2$	$17.5^{ab} \pm 1.6$	<0.01
C18:2TFA	$0.9^{b} \pm 0.2$	$0.8^{ab} \pm 0.2$	$0.6^{a} \pm 0.2$	$0.5^{a} \pm 0.2$	$0.6^{a} \pm 0.1$	$0.5^{a} \pm 0.2$	<0.01
C18:2*n*6	$1.5^{ab} \pm 0.4$	$1.2^{a} \pm 0.1$	$1.6^{b} \pm 0.2$	$1.5^{ab} \pm 0.4$	$1.1^{a} \pm 0.4$	$1.4^{ab} \pm 0.6$	<0.01
C18:3*n*3	$0.8^{b} \pm 0.2$	$0.5^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.7^{ab} \pm 0.2$	$0.5^{a} \pm 0.1$	$0.5^{ab} \pm 0.2$	<0.01
C18:3TFA	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.4$	$0.4^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	
C18:3*n*6	$0.2^{b} \pm 0.0$	$0.1^{ab} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{ab} \pm 0.0$	$0.2^{ab} \pm 0.0$	$0.2^{ab} \pm 0.0$	<0.05
C18:4*n*3	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	
C19:0	$0.5^{b} \pm 0.1$	$0.4^{ab} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.4^{ab} \pm 0.1$	<0.01
C20:0	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	
C20:2*n*6	$0.0^{a} \pm 0.1$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	
C20:3*n*6	$0.0^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	
C20:3*n*3	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	
C20:4*n*6	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	<0.05
C20:5*n*3	$0.1^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.1^{ab} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	<0.01
C22:0	$0.1^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{ab} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	<0.01
C22:2*n*6	$0.0^{bc} \pm 0.0$	$0.1^{c} \pm 0.0$	$0.0^{abc} \pm 0.0$	$0.0^{abc} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{ab} \pm 0.0$	<0.01
C24:0	$0.1^{a} \pm 0.0$	$0.0^{ab} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{ab} \pm 0.0$	$0.0^{ab} \pm 0.0$	$0.0^{ab} \pm 0.0$	<0.01
CLA	$1.1^{c} \pm 0.2$	$0.7^{b} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.5^{ab} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	<0.01
SFA	$65.2^{a} \pm 2.1$	$67.1^{ab} \pm 0.9$	$67.9^{abc} \pm 1.7$	$70.0^{c} \pm 2.37$	$69.1^{bc} \pm 2.5$	$69.1^{abc} \pm 3.0$	<0.01
MUFA	$26.57^{b} \pm 1.55$	$26.58^{b} \pm 2.01$	$25.92^{b} \pm 1.47$	$21.6^{a} \pm 2.3$	$22.5^{a} \pm 2.2$	$22.4^{a} \pm 1.8$	<0.01
PUFA	$4.9^{b} \pm 0.5$	$3.9^{a} \pm 0.5$	$3.8^{a} \pm 0.418$	$4.0^{ab} \pm 0.8$	$3.4^{a} \pm 0.5$	$3.6^{a} \pm 1.2$	<0.01

(SOM) Summer organic milk; (SPM) Summer pasture milk; (SCM) Summer conventional milk; (WOM) Winter organic milk; (WPM) Winter pasture milk; (WCM) Winter conventional milk; (TFA) *Trans* fatty acid; (CLA). Conjugated linoleic acids; (SFA).Saturated fatty acid; (MUFA).Monounsaturated fatty acid (PUFA).Polyunsaturated fatty acid; $^{a-c}$ Means with different superscripts are significantly different (P<0.05) according to the Mann-Whitney U-test.



559

560 **Fig. 1.** Principal component analysis (PCA) score and loadings plots of the first two principal
561 components of the fatty acid compositions of different forages obtained from the European summer and
562 winter periods: (a) summer forages PCA biplot; (b) winter forages PCA biplot; (c) overall forages PCA
563 scores plot.

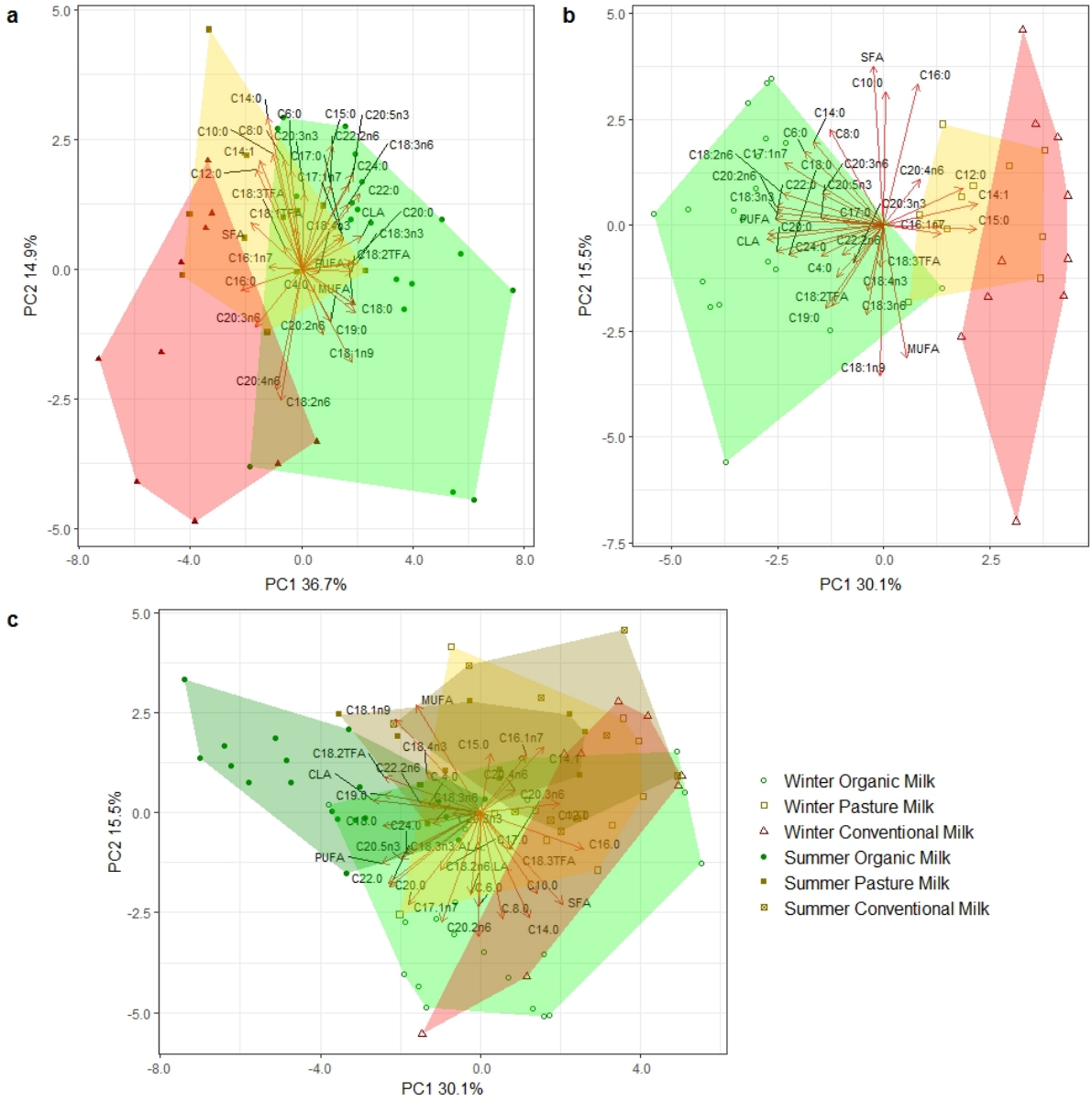
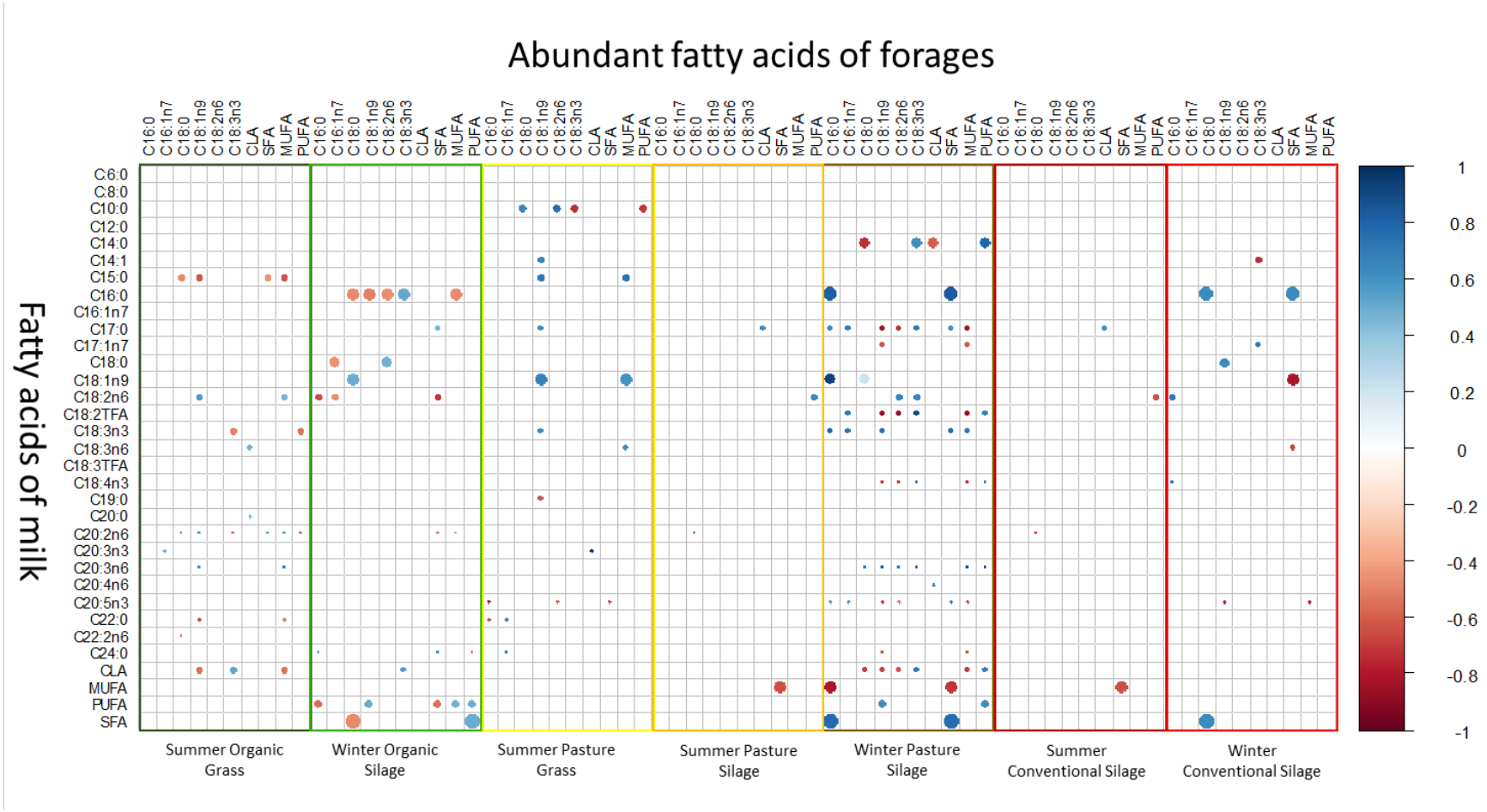


Fig. 2. Principal component analysis (PCA) scores and loadings plots of the first two principal components of the fatty acid compositions of different types of milk obtained from the European summer and winter periods: (a) summer milk PCA biplot; (b) winter milk PCA biplot; (c) PCA biplot of summer and winter milks.



570

571 **Fig. 3.** The significant ($P < 0.05$) Pearson correlation coefficients (r) for the fatty acids of the milk vs. the most abundant fatty acids of the respective forage

572 types. The different colours of the symbols indicate the different positive (blue) and negative (red) coefficient values and the different sizes of the symbols relate

573 to the different concentrations of the fatty acids in the milk.