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Potentials to differentiate milk composition by different feeding strategies

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ABSTRACT

To investigate the effect of the dietary intake of the cow on milk composition, bulk-tank milk was collected on 5 occasions from conventional (n = 15) and organic (n = 10) farms in Denmark and on 4 occasions from low-input nonorganic farms in the United Kingdom, along with management and production parameters. Production of milk based on feeding a high intake of cereals, pasture, and grass silage resulted in milk with a high concentration of α-linolenic acid (9.4 ± 0.2 mg/kg of fatty acids), polyunsaturated fatty acids (3.66 ± 0.07 mg/kg of fatty acids), and natural stereoisomer of α-tocopherol (*RRR*-α-tocopherol, 18.6 ± 0.5 mg/kg of milk fat). A milk production system using a high proportion of maize silage, by-products, and commercial concentrate mix was associated with milk with high concentrations of linoleic acid (LA; 19.7 ± 0.4 g/kg of fatty acids), monounsaturated fatty acids (27.5 ± 0.3 mg/kg of fatty acids), and a high ratio between LA and α-linolenic acid (4.7 ± 0.2). Comparing these 2 production systems with a very extensive nonorganic milk production system relying on pasture as almost the sole feed (95 ± 4% dry matter intake), it was found that the concentrations of conjugated LA (*cis*-9,*trans*-11; 17.5 ± 0.7 g/kg of fatty acids), *trans*-11-vaccenic acid (37 ± 2 g/kg of fatty acids), and monounsaturated fatty acids (30.4 ± 0.6 g/kg of fatty acids) were higher in the extensively produced milk together with the concentration of antioxidants; total α-tocopherol (32.0 ± 0.8 mg/kg of milk fat), *RRR*-α-tocopherol (30.2 ± 0.8 mg/kg of milk fat), and β-carotene (9.3 ± 0.5 mg/kg of milk fat) compared with the organic and conventional milk. Moreover, the concentration of LA (9.2 ± 0.7 g/kg of fatty acids) in milk from the extensive milk production system was found to approach the recommended unity ratio between n-6 and n-3, although extensive milk production also resulted in a lower daily milk yield.

Key words: organic milk, dairy cow diet, ratio between n-6 and n-3, antioxidant

INTRODUCTION

In recent years, the demand for food with high nutrient value and high sensory quality has increased. Linoleic acid (**LA**) and α-linolenic acid (**ALA**) are essential fatty acids because they cannot be synthesized by the human body (Stark et al., 2008), and they are the major n-6 and n-3 fatty acids in milk. Several studies indicate that ALA has cardio-protective effects (Albert et al., 2005; Djoussé et al., 2005). In recent years, the consumption of n-6 fatty acids like LA has risen dramatically in developed nations. Thus, according to Simopoulos (2002), consumers should lower their intake of n-6 fatty acids and increase the intake of n-3 fatty acids, so the ratio between these 2 fatty acids approaches the optimal 1. In addition, it has been stated that an increase of conjugated linoleic acids (**CLA**) in the human diet is beneficial to health (Bhattacharya et al., 2006). However, the beneficial effects are proven in animals and not yet in humans, but the effect of CLA on human health is still in focus. The fatty acid distribution in milk fat is dependent on dietary composition (Dewhurst et al., 2003b). For example, pasture-based diets supply a significantly higher level of unsaturated fatty acids compared with TMR diets with roughage based on maize (*Zea mays*), and this is reflected in a higher concentration of unsaturated fatty acids in the milk fat (Bargo et al., 2006; Couvreur et al., 2006; Croissant et al., 2007). Notably, maize silage contains a high concentration of LA, which affects the milk composition with an increase particularly in this n-6 fatty acid (Walker et al., 2004). Therefore, changing the feeding for the dairy cow will modify the composition of the fatty acids in milk, especially the polyunsaturated fatty acids (**PUFA**). An increased proportion of unsaturated fat in milk may increase the oxidative susceptibility of milk, and to maintain a high quality, the concentration of antioxidants should therefore also be elevated. In milk, the concentration of α-tocopherol and carote-

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noids as antioxidants is believed to be important for the oxidative stability; tocopherol and β-carotene scavenge lipid peroxy radicals and quench singlet oxygen. However, there are contradictive results in this field because earlier work shows prooxidative behavior of α-tocopherol, when no coantioxidant, such as coenzyme Q, is present (Thomas et al., 1996), or when a high concentration of unsaturated fat and α-tocopherol is present in milk (Slots et al., 2007). High concentrations of α-tocopherol and β-carotene in milk can be obtained by a high proportion of pasture or grass clover silage in the feed of dairy cows (Havemose et al., 2004) because these forage types are high in antioxidants (Lynch et al., 2001). Together, these results indicate that the composition of milk can be manipulated to become closer to recent Danish dietary recommendations for humans and thus have higher content of PUFA and vitamins by increasing reliance on pasture-based diet for the cows. It is fundamental to study how herd-level management and nutritional factors affect the composition of the produced milk to formulate recommendations for producers aiming to enhance important components in milk. Hence, the aim of this study was to find out if the composition of milk produced by dairy farmers could be differentiated by the management at the dairy farm.

MATERIALS AND METHODS

Chemicals and Materials

The tocopherol standards, *all-rac*-α-tocopherol standard, *RRR*-α-tocopherol standard, carotenoid standards, fatty acid methyl esters, and butyl hydroxyl toluene (99.0%), were obtained from Sigma (Sigma Chemical Co., St. Louis, MO); methanol (HPLC-grade) and sodium chloride from J. T. Baker (Deventer, Holland); sodium hydroxide from Merck (Merck KGaA, Darmstadt, Germany); potassium hydroxide and sodium metal for producing sodium methylate from Merck-Schuchardt (Merck-Schuchardt & Co., Hohenbrunn, Germany); *n*-heptane, chloroform, and 2-propanol (all HPLC-grade) from LabScan (LabScan Ltd., Stilloragan Industrial Park, Co. Dublin, Ireland); *n*-hexane, pentane, dichloromethane and acetonitrile (all HPLC-grade) from Rathburn (Rathburn Chemicals Ltd., Walkerburn, Scotland); ethylene glycol dimethyl ether (HPLC-grade) and dimethyl sulfate (99+%) from Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany); triethylamine (≥99.5%) from Fluka (Sigma-Aldrich Chemie GmbH); ethanol (96% vol/vol) from The Danish Distillers (Copenhagen, Denmark); and the water used was purified through an Elgastat Maxima unit (Bucks, UK) before use.

Experimental Setup

Milk samples were collected from commercial dairy herds covering 3 milk production systems: conventional and organic milk production from Denmark and an extensive milk production from the United Kingdom. Using a standard questionnaire, management and production parameters were recorded 5 times during 1 yr from the 2 milk production systems in Denmark and 4 times from the extensive milk production system. The reason for only 4 recordings from the extensive production system was the use of spring block calving, and the dairy cows were therefore not lactating from November to February. In the Danish production systems, all herds had all year-round calving.

The feed intake for the organic and conventional milk production systems including the pasture intake was calculated by the research technician according to Danish standards based on the difference between herd demand and recorded intake of supplements, whereas the pasture intake for the extensive milk production system was calculated as the difference between an estimated total intake of 16.9 kg of DM and the recorded intake of conserved forage and concentrates. Furthermore, the amounts of conserved forage [separated into maize silage, grass silage, other silages (primarily barley whole-crop silage), and hay-straw] and concentrate (separated into cereals, by-products, and commercial concentrate mix) were registered at herd level (Table 1). The use of additional vitamins as supplement to the feed was recorded in the standard questionnaire as a used-not used option. Milk production was recorded at herd level as amount of milk delivered, and the content of fat was taken from the information given by the dairy company. Samples of milk were taken from stirred bulk tanks after 2 milkings at each farm, which represented 24 h of production, and it was frozen immediately after sampling and kept at −20°C until analysis. In the statistical data analysis, the feed intakes are averaged over the year.

Conventional Milk Production Systems. Fifteen conventional dairy Holstein herds representing the common conventional milk production system in Denmark were selected, and in the diets they predominantly used a commercial concentrate mix (dominated by citrus pulp, rapeseed, and soybean meal), by-products (such as dried sugar beet pulp), and conserved forage, primarily maize silage and grass silage from ryegrass fields.

Organic Milk Production Systems. Ten organic dairy Holstein herds representing the common certified organic milk production system were selected. They produced milk according to the legislation concerning organic farming issued by the Danish Ministry of Food,

Table 1. Composition of DMI from conventional (CPS), organic (OPS), and extensive milk production systems (EPS)[1]

Item	CPS	OPS	EPS	df	GLM[2] (*P*-value)
n[3]	15 (75)	10 (50)	5 (18)		
Feed intake (% of DMI)					
Pasture	4 ± 2[c]	16 ± 2[b]	94 ± 4[a]	2	<0.0001
Concentrate[4]	38 ± 1[a]	31 ± 1[b]	6 ± 2[c]	2	<0.0001
Cereals	4.2 ± 0.6[b]	17.3 ± 0.7[a]	0.2 ± 1.2[c]	2	<0.0001
By-products[5]	11.2 ± 0.9[a]	1.1 ± 1.2[b]	3.7 ± 1.9[b]	2	<0.0001
Commercial concentrates mix	23 ± 1[a]	13 ± 1[b]	2 ± 2[c]	2	<0.0001
Conserved forage[6]	55 ± 2[a]	52 ± 2[a]	0 ± 4[b]	2	<0.0001
Grass silage	18 ± 2[b]	33 ± 2[a]	0 ± 4[c]	2	<0.0001
Maize silage	32 ± 1[a]	10 ± 2[b]	0 ± 3[c]	2	<0.0001
Other silages	6 ± 1[a]	9 ± 2[a]	0 ± 3[b]	2	0.0151
Hay-straw	1.7 ± 0.3[a]	0.9 ± 0.3[ab]	0 ± 0.6[b]	2	0.0132

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Means ± standard error.

[2]GLM = general linear model in SAS (SAS Institute Inc., Cary, NC).

[3]n = the number of dairy herds and samples (in parentheses) in each production system.

[4]Concentrate = sum of cereals, by-products, and commercial concentrate mix.

[5]By-product consists mainly of brewers and distillers waste or sugar beet pulp, or both.

[6]Conserved forage = sum of grass silage, maize silage, and other silages.

Agriculture and Fisheries. Together with the Council Regulation no. 2092/91 (Council Regulation, 1991), this legislation regulates organic cow management and feeding. The organic dairy cows in Denmark must be on pasture for at least 150 d a year between the April 15 and the November 1 (Ministry of Food, Agriculture and Fisheries, 2007). The organic milk producers predominantly use pasture and grass silage from white clover-ryegrass fields and cereals (oat and barley) in the feed. The milk samples in both systems were taken in April, June, September, December, and February at all farms.

Extensive Milk Production Systems. Five dairy herds (a mix of Holstein-Friesian and Jersey) representing the extensive milk production systems in the United Kingdom were selected. All farms used spring block calving, where cows were grazed during lactation. Either no concentrates or only up to 2 kg of DM per cow daily were given as supplement to pasture (white clover-ryegrass). The cows were only housed when not lactating between November and February. The milk samples were taken in August, October, March, and May at all farms.

Extraction and Quantification Methods

Extraction, Separation, and Quantification of Tocopherols from Milk. Extraction, separation, and quantification of tocopherols from the milk were performed as described by Havemose et al. (2004) with modifications as explained in Slots et al. (2007). Milk (2.00 mL) and acetic acid in ethanol [2.00 mL 1% (wt/vol)] were mixed for 10 s. Saturated potassium hydroxide in water (0.30 mL) was added. The dispersion was mixed for 10 s and subsequently heated to 70°C for 60 min. The saponified dispersion was cooled on ice, and demineralized water (1.00 mL) and heptane (3.00 mL) were added. The dispersion was mixed again for 1 min and centrifuged for 3 min at 1,700 × *g*. The upper layer was isolated and filtered into an HPLC vial for separation and quantification. Separation of the tocopherols was performed on an HPLC system (HP 1100, Hewlett-Packard Co., Santa Clara, CA) with a Hypersil Si column (25 × 0.4 cm; Hewlett-Packard Co., Santa Clara, CA). The injection volume was 30 μL, the mobile phase *n*-hexane:2-propanol (100:2 vol/vol), and the flow rate 1.0 mL/min. Detection was performed by fluorescence with excitation at 295 nm and with emission at 330 nm. Quantification was performed by use of external standards.

Isomeric Distribution of Extracted Tocopherol Based on Derivatization to Tocopheryl Methyl Ether. Derivatization and isomeric distribution of α-tocopherol stereoisomers to the corresponding α-tocopheryl methyl ether stereoisomers were performed as described by Riss et al. (1994) with modification as explained in Slots et al. (2007). The extracted tocopherol mixture (2.00 mL) was evaporated under nitrogen to dryness and dissolved in *n*-heptane (500 μL). Ethylene glycol dimethyl ether (200 μL) was added, and the solution was stirred during the whole derivatization procedure. Saturated sodium hydroxide (100 μL) was added drop-wise to the tocopherol mixture followed by addition of dimethyl sulfate (120 μL). The headspace over the samples was replaced by argon, and the samples were incubated for 1 h at room temperature.

After incubation, dimethyl sulfate (60 μL) was added, and the samples were incubated for 2 h at room temperature. Subsequently, they were dried under nitrogen at 40°C. Demineralized water (400 μL) was added to the dried sample, and the α-tocopheryl methyl ethers were extracted twice using *n*-hexane (2.00 mL). The organic phase containing α-tocopheryl stereoisomers was separated from the aqueous phase by centrifugation at 1,700 × *g* for 5 min and subsequently concentrated to 1 mL by evaporation before HPLC analysis. Separation of the stereoisomers of α-tocopheryl methyl ethers from milk and feed was performed by HPLC (HP 1100 series, Hewlett-Packard Co.) with a Chiralcel OD column (25 × 0.46 cm; Daicel Chemical Industries Ltd., Illkirch, France). The injection volume was 100 μL, the mobile phase was *n*-hexane, and the flow rate was 1.0 mL/min. Detection was performed by fluorescence detection with excitation at 291 nm and emission at 330 nm. Identification of the specific isomers was based on external standards for *RRR*-α-tocopherol and *all-rac*-α-tocopherols as described by Riss et al. (1994).

Extraction and Detection of Carotenoids from Milk. Extraction from and detection of carotenoids in milk was performed as described by Havemose et al. (2004) with modifications. Ethanolic butylhydroxytoluene (2 mL, 1% wt/vol) was added to milk samples (2 mL) and mixed for 10 s. A saturated potassium hydroxide solution (0.5 mL) was added, and the samples were mixed again for 10 s. The headspace above the samples was replaced with argon, and the samples were incubated for 60 min at 70°C. Subsequently, the samples were cooled on ice and 1 mL of water was added. The carotenoids were extracted first with 3 mL of heptane:dichloromethane (100:10 vol/vol) and afterwards twice with heptane:dichloromethane (100:20 vol/vol). In between the extraction steps, the samples were centrifuged for 3 min at 1,700 × *g*, and the supernatant was collected. The extracts were evaporated under nitrogen at 50°C until dryness and redissolved in 2 × 0.5 mL of a mixture of acetonitrile:methanol:dichloromethane (100:10:5 vol/vol/vol) with further addition of triethylamine (0.5 mL/L). Separation of the carotenoids was done with 100 μL of the extract into an HPLC system (HP 1100, Agilent Technologies, Palo Alto, CA) with diode array detection at 448 nm for lutein and 455 nm for β-carotene and zeaxanthine. The flow of the mobile phase on the HPLC system was 1.6 mL/min for the first 7 min and afterward increased to 2.2 mL/min to a final run time of 22 min. The primary column was a Vydac Protein Peptide column (The Separations Group Inc., Hesperia, CA), and the secondary column was a Hypersil octadecyl silane column (Agilent Technologies). The mobile phase was similar to the mixture used to redissolve the extracted carotenoids. The carotenoids were quantified using external standards.

Extraction and Detection of Fatty Acids in Milk. Extraction from methylation and detection of fatty acids in milk was performed as described by Havemose et al. (2004) and by Slots et al. (2007) with modification. Milk fat was extracted from milk by adding methanol (2.00 mL) and chloroform (4.00 mL) to milk (2.00 mL). This mixture was firmly mixed for 1 min followed by centrifugation for 10 min at 3,000 × *g*. The lower chloroform phase containing the lipid fraction was isolated. This extraction procedure was repeated twice giving a total of 3 extraction steps to determine the total fat concentration in the sample. Methylation of the fatty acids was done by evaporating 2 mL of the chloroform phase to dryness under nitrogen. Fat (approximately 10 mg) was dissolved in sodium methanolate (1.00 mL), and the tube was filled with argon and closed. After incubation at 60°C for 30 min and subsequent cooling on ice, saturated sodium chloride solution (4.00 mL) and pentane (1.00 mL) were added. The samples were mixed on a vortex mixer for 1 min and centrifuged at 1,700 × *g* for 10 min. The pentane phase with the fatty acid methyl esters (2.0 μL) was injected to a GC system (HP6890 gas chromatography system, Hewlett-Packard Co) with a flame-ionization detector and an HP RT-2560 column (100 m × 0.25 mm × 0.2 μm). The inlet temperature was 275°C, and the carrier gas was helium with a constant flow of 1.5 mL/min. The starting temperature at 40°C was held for 2 min and then increased by 4°C/min to 165°C and held there for 5 min. Subsequently, the temperature was increased by 5°C/min to an end temperature of 220°C, held for 10 min, and again increased by 4°C/min to an end temperature of 240°C, which was held for 10 min giving a total run time of approximately 50 min. Equilibration time was 1.0 min. The detector temperature was 300°C, and split ratio and split flow were 40:1 and 60.0 mL/min, respectively. Identification was based on external standards, and calculation of the distribution (in weight percentage) was based on the area of each fatty acid ester corrected for the response factors for the individual fatty acids. Internal standards were used to determine percentage of recovery.

Statistical Analysis

Data were analyzed by principal component analysis (**PCA**) and by partial least squares regression analysis (**PLS**) in Unscrambler (version 9.6, Camo Software A/S, Oslo, Norway) to determine how the feed of the dairy cows and the composition of the milk were differentiated from each other. Principal component analysis

reduces the number of variables in a multidimensional data set to lower dimensions for analysis, and it retains a set of principal components (**PC**) that capture the most related variation. The loadings represent coefficients of the PC and reflect both how much the original variables contribute to PC and how well PC take into account variation of a variable over the data points. Variables located close to each other are correlated, whereas variables located opposite each other are negatively related. The variables located close to origin contribute less to the explanation of the variation in the samples. Partial least squares regression analysis finds a linear model that relates the variations in 1 or several response variables (Y-variables) to the variations of several predictors (X-variables), for explanatory purposes. The response variables (Y-variables) analyzed by PLS in the present study are the concentrations of linoleic acid, linolenic acid and α-tocopherol in milk. The X-variables consisted of the 8 feed variables (pasture, grass silage, maize silage, other silage, hay or straw, cereals, by-products, and commercial concentrate mix). When analyzing the concentration of α-tocopherol in milk, the vitamin supplement was included as an X-variable together with the other feed variables. The GLM was conducted in SAS (version 9.1, SAS Institute Inc., Cary, NC) to explore the effect of the different variables. The mean value for the different variables was an average of all milk samples with no regards to the different sampling times, and the mean value was used directly in the PCA plot.

RESULTS

In the present study, production data collected from 25 farms show a large variation in feed intake between and within the 3 milk production systems. The feed intake from the organic and conventional herds was analyzed by PCA in Unscrambler. The first principal component (**PC1**) explained 41% of variation, and the second principal component (**PC2**) explained 14% of variation (Figure 1). In this loading plot, 2 groups could be distinguished: conventional showing negative loadings for PC1 and organic showing positive loadings for PC1, whereas the loadings for PC2 divided grass silage, showing positive loadings, from pasture and other silages, showing negative loadings. Maize silage, by-products, hay-straw, commercial concentrate mix, and vitamin supplement showed negative loadings for PC1 but were not distinguished by PC2. The differences found by the PCA were confirmed by ordinary statistical analysis (Table 1). Also, the milk composition of the organic and conventional production system was analyzed by PCA, and PC1 explained 26% and PC2 20% (Figure 2). Again, 2 groups could be distinguished: milk from the organic dairy herds had a significantly higher concentration of ALA and natural stereoisomer of α-tocopherol (*RRR*-α-tocopherol) in the milk (Figure 2), which is confirmed in Table 2. The concentration of ALA, PUFA, and *RRR*-α-tocopherol was 51, 10, and 13% higher, respectively, in the organic milk compared with the conventional milk (Table 2). Milk from conventional dairy herds contained a 12, 6, and 60% higher concentration of LA, monounsaturated fatty acids (**MUFA**), and a high ratio between n-6 (LA) and n-3 (ALA) in the milk compared with the organic milk (Table 2). The concentration of saturated fatty acids (**SAT-FA**) was 7 and 5% lower in milk from the extensive production system compared with milk from the organic and conventional production systems, respectively, and the dairy cows in the conventional milk production system had 16% higher daily milk yield compared with the dairy cows in the organic milk production system. When comparing the above 2 milk production systems with a very extensive milk production system using almost only pasture (95% of total intake) in the diet of the dairy cows (Table 1), the concentrations of CLA (*cis*-9,*trans*-11), *trans*-11-vaccenic acid, and MUFA in the milk (Table 2) were 53, 41, and 15% higher, respectively, for milk from the extensive milk production compared with milk from the organic milk production and 61, 46, and 10%, respectively, compared with milk from the conventional production system. Furthermore, the concentrations of the antioxidants α-tocopherol and β-carotene in the milk, which are both supposed to be protective against oxidation of milk fat, were 34 and 54% higher in milk from the extensive production system compared with milk from the organic and conventional systems, respectively (Table 2). Also, the concentration of milk fat was higher in the extensive production system compared with the 2 other production systems. However, the daily milk yield of dairy cows was 27 and 39% lower in the extensive management system compared with the organic and conventional management systems, respectively (Table 2). Overall, a diet high in pasture results in lower concentrations of SAT-FA and higher concentrations of MUFA, PUFA, and antioxidants in milk compared with a diet low in pasture. Upon a comparison of the organic milk production systems with the extensive system, the concentration of ALA in the organic milk was not found to be different from the concentration in milk from the extensive milk production system (Table 2). Accordingly, a PLS analysis was performed to specify the feed components that significantly increase the concentration of ALA in the organic and conventional milk. The concentration of ALA was the response variable (Y-variable; the measured output variable that describes the outcome of the experiment), and the amounts of

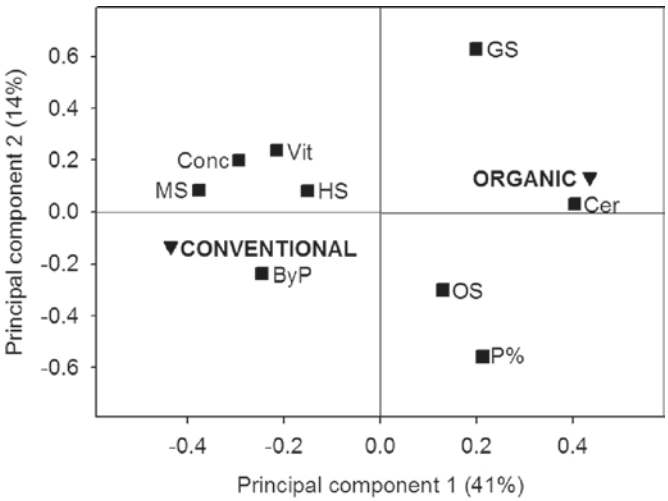

Figure 1. Principal component analysis loading plot for milk samples from conventional and organic milk production systems (▼) and the different food types (■) used in the diet. ByP = proportion of by-products in diet; Cer = proportion of cereals in diet; Conc = proportion of commercial concentrate mix in diet; GS = proportion of grass silage in diet; HS = proportion of hay or straw in diet; MS = proportion of maize silage in diet; OS = proportion of other silages in diet; P% = percentage of pasture in diet; Vit = vitamin supplement.

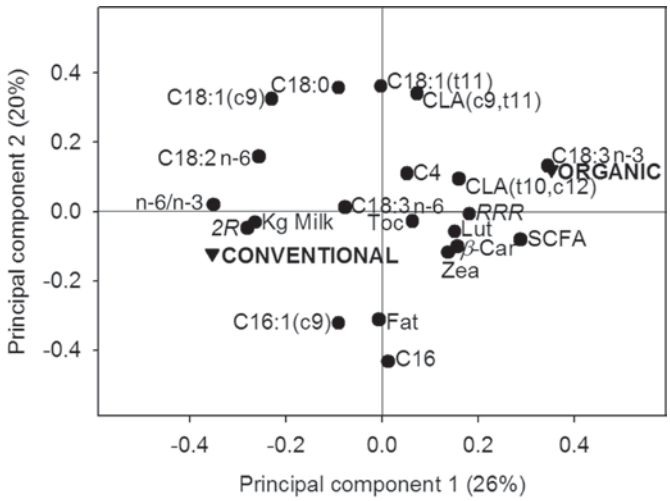

Figure 2. Principal component analysis loading plot for milk samples from conventional and organic milk production systems (▼) and the different constituents in the organic and conventional milk (●). β-Car = concentration of β-carotene in milk; Kg Milk = kilograms of milk produced per cow; Lut = concentration of lutein in milk; *RRR* = amount of *RRR*-α-tocopherol in milk; *2R* = amount of synthetic *2R*-α-tocopherol in milk; SCFA = concentration of sum of short-chain fatty acids (C6 to C14) in milk; Toc = concentration of α-tocopherol in milk; Zea = concentration of zeaxanthine in milk; C4 = concentration of butyric acid in milk; C16 = concentration of palmitic acid in milk; C16:1 (c9) = concentration of palmitoleic acid in milk; C18:0 = concentration of stearic acid in milk; C18:1(c9) = concentration of oleic acid in milk; C18:1(t11) = concentration of *trans*-11-vaccenic acid in milk; C18:2n-6 = concentration of linoleic acid in milk; CLA(c9,t11) = concentration of conjugated linoeic acid (*cis*-9,*trans*-11) in milk; CLA(t10,c12) = concentration of conjugated linoeic acid (*trans*-10,*cis*-12) in milk; C18:3n-3 = concentration of α-linolenic acid in milk; C18:3n-6 = concentration of γ-linolenic acid in milk; n-6/n-3 = n-6:n-3 fatty acid ratio in milk.

the 8 feed variables were predictors for the organic and conventional milk production systems (X-variables; Figure 3A). The regression coefficients (the numerical coefficients that express the link between variation in predictors and variation in response) were significant for the proportion of cereals, pasture, and grass silage in the feed, indicating that these feed components increase the concentration of ALA in milk from the organic and conventional milk production systems. The proportion of maize silage, other silages, by-products, and commercial concentrate mix in the feed gave in contrast a lower ALA concentration in the organic and conventional milk. Moreover, the concentration of LA was low in milk from the extensive milk production system, and to identify which feed components in the organic and conventional milk production systems, which had an effect on the concentration of LA in milk, a PLS analysis was performed with the concentration of LA in the milk as response variable and the 8 feed variables as predictors (Figure 3B). In this case, the regression coefficients were only significant for the proportion of commercial concentrate mix in the diet, indicating that use of commercial concentrate mix increases the content of LA in organic and conventional milk. However, the proportion of grass silage and other silages in the feed resulted in a low concentration of LA in the milk from the 2 production systems. A PLS analysis for the effect of the 8 feed components together with vitamin supplement (predictors) on the concentration of α-tocopherol in milk (response variable) from the organic and conventional milk production systems showed that the regression coefficient was only significant for the proportion of cereals and by-products in the feed, whereas the proportion of by-products in the feed resulted in a low concentration of α-tocopherol (Figure 4).

DISCUSSION

This study aims to show the potential of herd-level management and feeding of the dairy cows to affect the composition of milk. When grazing pasture, the concentration of SAT-FA was lowered in the milk from the extensive production system, and possibly the de novo synthesis of fatty acids in the mammary gland for cows grazing pasture decreased. Unsaturated fatty acids present in the diet or originating from the mobilization of body reserves exert a potent inhibitor effect on the de novo synthesis at the mammary gland, which is responsible for the decrease in the synthesis of short-

Table 2. Daily milk yield, fat concentration, fatty acid distribution, ratio between n-6 and n-3 fatty acids, and the concentration of fat-soluble antioxidants in milk samples from conventional (CPS), organic (OPS), and extensive milk production system (EPS)[1]

Item	CPS	OPS	EPS	Degrees of freedom	GLM[2] (*P*-value)
n[3]	15 (75)	10 (50)	5 (17)		
Daily milk yield (kg of milk/cow per day)	29.3 ± 0.3[a]	24.6 ± 0.4[b]	17.9 ± 0.6[c]	2	<0.0001
n	75	50	18		
Fat concentration (g/100 g of milk)	4.12 ± 0.03[b]	4.05 ± 0.04[b]	4.57 ± 0.06[a]	2	<0.0001
Fatty acids[4] (g/kg of fatty acids)					
n	75	50	20		
CLA (*cis*-9,*trans*-11)	6.8 ± 0.4[b]	8.2 ± 0.5[b]	17.5 ± 0.7[a]	2	<0.0001
Trans-11-vaccenic acid	20 ± 1[b]	22 ± 1[b]	37 ± 2[a]	2	<0.0001
ALA	4.6 ± 0.2[b]	9.4 ± 0.2[a]	9.0 ± 0.4[a]	2	<0.0001
LA	19.7 ± 0.4[a]	17.3 ± 0.5[b]	9.2 ± 0.7[c]	2	<0.0001
SAT-FA	692 ± 3[a]	706 ± 4[a]	659 ± 6[b]	2	<0.0001
MUFA	275 ± 3[b]	258 ± 4[c]	304 ± 6[a]	2	<0.0001
PUFA	33.1 ± 0.6[b]	36.6 ± 0.7[a]	37.0 ± 1.2[a]	2	0.0003
n-6:n-3 ratio	4.7 ± 0.2[a]	1.9 ± 0.2[b]	1.0 ± 0.3[c]	2	<0.0001
Fat-soluble antioxidants (mg/kg of milk fat)					
n	75	50	20		
α-tocopherol	20.3 ± 0.4[b]	21.0 ± 0.5[b]	32.0 ± 0.8[a]	2	<0.0001
RRR-α-tocopherol	16.2 ± 0.4[c]	18.6 ± 0.5[b]	30.2 ± 0.8[a]	2	<0.0001
β-carotene	3.7 ± 0.2[b]	4.3 ± 0.3[b]	9.3 ± 0.5[a]	2	<0.0001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Means ± standard error.

[2]GLM = general linear model in SAS (SAS Institute Inc., Cary, NC).

[3]n = the number of dairy herds and samples (in parentheses) in each production system.

[4]CLA = conjugated linoleic acid; ALA = α-linolenic acid; LA = linoleic acid; SAT-FA = saturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids.

chain fatty acids and to a lower extent of medium-chain fatty acids (Barber et al., 1997). In the present study, pasture and grass silage in the diet contributed to a higher concentration of ALA in the milk, whereas maize silage contributed to a lower concentration of ALA. The pasture eaten by the cow in both the extensive production system and the organic system consisted of white clover and ryegrass, and becausae white clover contains a high proportion of ALA (Dewhurst et al., 2003a), this could be a reasonable explanation for the high level of ALA in the milk. A lower rate of hydrogenation due to a change in the rumen ecosystem cannot be excluded either (Chilliard et al., 2001). The low level of LA in milk from the extensive milk production system seems to be caused by a restricted use of maize silage and cereals because maize silage is rich in LA, because maize grain, which represents 30 to 40% of silage, comprises approximately 60% LA (Chilliard et al., 2001). The combination of the different forage types used in the organic milk production system in the present study was found to increase the concentration of ALA in the milk to the same extent as in the extensive production, which enables achievement of the favorable ratio of 1 between n-6 and n-3 fatty acids (Simopoulos, 2002) in the organic milk. However, improvement in the n-6:n-3 ratio could also be achieved by lowering the concentration of LA through change of feed. An elevated concentration of unsaturated fatty acids, especially PUFA in milk from the organic and extensive production system, carries the risk of oxidative instability, which, however, may be controlled by a high concentration of natural antioxidants in the milk. In the present study, the concentration of especially the natural stereoisomer of α-tocopherol (*RRR*-α-tocopherol) and β-carotene in the milk was high when the cows had received high levels of pasture or grass silage. This could be due to higher levels of α-tocopherol and β-carotene in pasture than in maize silage and cereals (Havemose et al., 2004), although perhaps the estimation of pasture intake may not be sensitive enough to detect differences in intake between systems. The lower daily milk yield from cows receiving high pasture intakes could also explain the higher concentration of α-tocopherol and β-carotene in the milk due to less dilution (Ellis et al., 2007). However, the dynamics of α-tocopherol and β-carotene transfer to milk are in general not well understood, and it is rather puzzling that the concentration of α-tocopherol and β-carotene in milk in the present study was not affected by the proportion of pasture used in the organic and conventional milk production systems. We suggest that the dilution effect of α-tocopherol could be due to a continuing development of breeding and management systems that focus solely on increasing milk and milk fat yield, in effect resulting in a constant dilution

of these vitamins/antioxidants in the milk fat as suggested by Jensen et al. (1999). The PLS model verifies the above because the model in Figure 4 is only explained by 3.5% ($R^2_{validated} = 0.0350$), indicating within this data set that α-tocopherol was not influenced through feeding. The vitamin supplement, mainly used in the conventional production system (Figure 1), gave a higher concentration of the synthetic stereoisomers of α-tocopherol in the conventional milk (indicated as *2R* in Figure 2), which is reasonable because the vitamin supplement consists of a racemic mixture of all of the 8 stereoisomers of α-tocopherol. Previously, work in our laboratory has shown that the cow discriminates between the stereoisomers of α-tocopherol because only the *2R* stereoisomers are excluded into the milk (Slots et al., 2007). The contents of CLA and *trans*-11-vaccenic acid in milk from the extensive production system were high compared with the other 2 systems and can be explained by high intakes of pasture by dairy cows under the extensive production system and to some extent in the organic system because lipid in herbage is high in PUFA. Generally, the milk fat content of CLA is affected by the intake of unsaturated fatty acids (Griinari et al., 1998), and in pasture, the major dietary unsaturated fatty acid is ALA, whereas CLA originates from the incomplete biohydrogenation of unsaturated fatty acids, especially LA, in the rumen. The ALA biohydrogenation in the rumen is known to not involve CLA as an intermediate, although *trans*-11-vaccenic acid is produced, and this is a known precursor of endogenous synthesis of CLA (*cis*-9,*trans*-11) in the lactating mammary gland (Griinari et al., 2000). Biohydrogenation of ALA will instead produce conjugated linolenic acid (Destaillats et al., 2005; Plourde et al., 2007), but conjugated linolenic acid was not measured in the present study. Factors that could have an effect on the production of CLA are the type, source, and level of carbohydrates, which influence the rates of microbial fermentation, or it could be the passage rate and fluid dilution rate, which increase during grazing because of the high water intake associated with grazing pasture (Kelly et al., 1998). Additionally, the variation in feed ration or the difference in breed and age of the studied animals may also have an effect on the concentration of CLA in milk (Stanton et al., 1997; Lawless et al., 1999). Also, the higher CLA concentrations insinuated in the PCA plot in organic milk compared with the conventional milk could be explained by the above. The many reports on the feeding of dairy cows between different breeds and countries are often in conflict, and direct comparison between countries must be considered problematic because the feeding strategies in the production systems are known to vary (e.g., in Sweden, the feeding strategies for organic and conventional milk

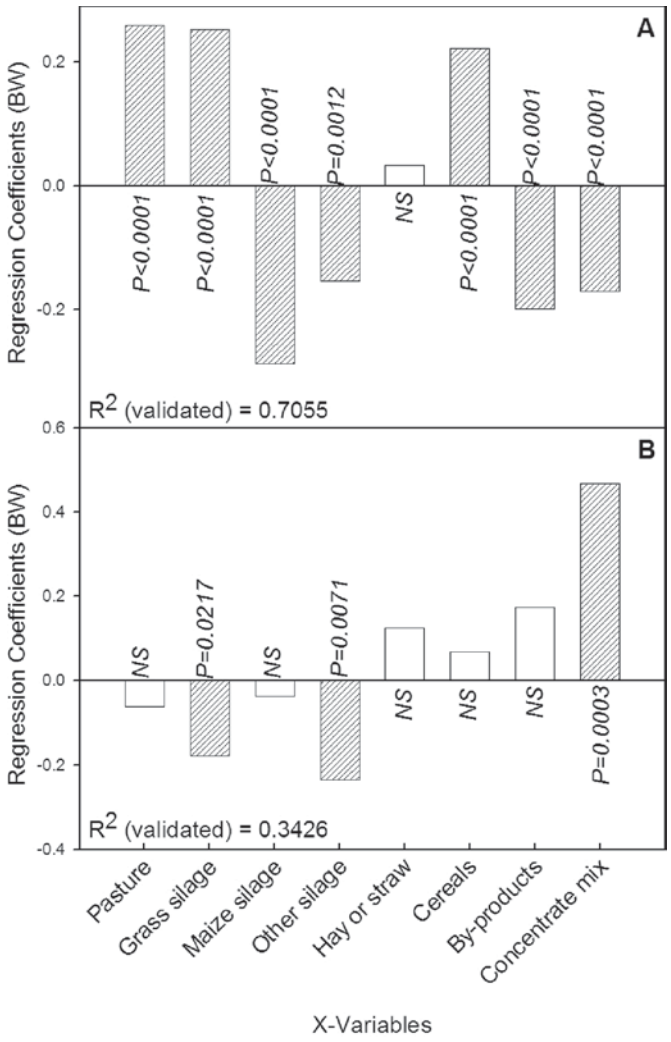

Figure 3. Regression coefficients (auto-scaled data) obtained by partial least squares regression analysis for A) the concentration of α-linolenic acid (C18:3n-3) in organic and conventional milk and B) the concentration of linoleic acid (C18:2n-6) in organic and conventional milk as response variable with the feed variables as predictors (X-variables) minus the vitamin supplement. The bars with diagonal stripes are significant.

production systems are very similar, whereas in Denmark, the conventional farmers use high levels of maize silage resulting in low concentrations of carotenoids in the milk, as maize silage in general is low in carotenoids; Noziere et al., 2006). However, in the present study, the dietary intake is recorded directly from the dairy farmers and hereby the variation between countries should be eliminated. Also, it seems fair to assume that the variation between breeds could be eliminated because a recent study showed no differences between Jersey and Holstein dairy cows in regard to the concentration of LA and ALA (Carroll et al., 2006). However, it is proposed that the breed affects the concentration of milk fat in the present study because Jersey cows are

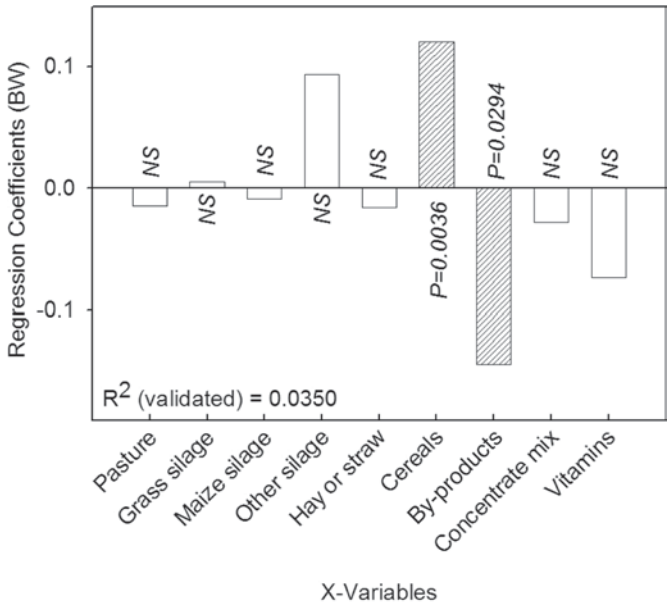

Figure 4. Regression coefficients (auto-scaled data) obtained by partial least squares regression analysis for the concentration of α-tocopherol in organic and conventional milk as response variable with the feed variables as predictors (X-variables). The bars with diagonal stripes are significant.

known to produce milk with a high concentration of fat compared with other cow breeds (Soyeurt et al., 2006). Therefore, the cross mix of Jersey and Holstein-Friesian in the herd of the extensive production system is probably the reason for the higher fat concentration in milk from EPS.

In conclusion, an extensive production system with a high contribution from pasture in the diet of the dairy cows results in milk with a higher concentration of ALA and a lower concentration of LA and accordingly a more favorable ratio between n-6 and n-3 compared with the organic and conventional milk. Moreover, the low daily milk yield in this system results in a higher concentration of antioxidants in the milk and probably in an improved oxidative stability of milk with such a high content of unsaturated fat. Therefore, an extensive production form with a high level of pasture is recommended for production of milk with a high content of PUFA and high levels of potential antioxidants.

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