Animal Science Papers and Reports vol. 34 (2016) no. 1, 25-40
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Alternative supplemental mixture for organic dairy herds to maintain desirable milk fatty acid profile throughout the indoor feeding period*

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(Accepted December 11, 2015)

The aim of the study was to evaluate the effect of diet containing a supplemental mixture with alternative components suitable for organic herds on the yield and composition of milk and the fatty acid profile. The on-farm experiment was carried out on 12 dairy cows (mean lactation 2.3, on average 116 days in milk) that were divided into 2 groups. During the experiment cows were fed on a diet based on maize silage (30 kg/d), lucerne hay (3 kg/d) and supplemental mixture (8 kg/d, all as fed basis). The control group (C) was fed a control supplemental mixture, while the experimental group (E) was fed a supplemental mixture that contained rapeseed and sunflower components. The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days (a 7-d preliminary period and a 7-d experimental period). The total content of SFA was higher in C in comparison to E (P<0.05). The content of C18:0 was higher, while contents of C12:0, C14:0 and C16:0 were lower in E compared to C (P<0.05). The total contents of UFA, MUFA and PUFA were higher in E than in C (P<0.05). The concentration of oleic acid (C18:1n9c) was considerably higher in E than in

* Supported by the Ministry of Agriculture of the Czech Republic, project NAZV No. QH81210 and NAZV KUS QJ1510336, the institutional support by decision RO1415 and by the Ministry of Education, Youth, and Sports of the Czech Republic, project No MSMT 6215712402.
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C (P<0.05). The atherogenic index (AI) and desaturation index (DI) calculated for E were lower than in C, while the spreadability index (SI) in E was higher in comparison with C (P<0.05).

KEYWORDS: dairy cows / milk fat / soybean / rapeseed cake / sunflower

One of the intentions of organic agriculture is to produce high-quality, nutritious food that contributes to human health and well-being [IFOAM, 2010]. The rules for organic milk production require a more roughage-based feed and no use of genetically modified or synthetic products.

Furthermore, the EC regulation [1999] requires that at least 60% dry matter (DM) in the daily ration have to consist of forage, or more specifically, the total daily dry matter proportion of concentrates should not exceed 50% during the first 3 months of lactation and thereafter it should be max. 40%. Additionally, the focus is on increasing the proportion of home-grown feed as one of the basic principles in organic farming [IFOAM 2002]. Apart from the crops that have typically been grown organically, such as grass-clover mixes for silage and cereals for maturity and for whole crop silage, in the last few years also rapeseed and lupins have been grown organically [Mogensen 2008] for incorporation to dairy rations. This is in contrast to conventional dairy farms that usually use imported soybean meal as a high-protein component to complement maize silage in the ration for dairy cows.

Previous studies on milk composition have shown that nutrient composition depends partly on the feeding regime [Palmquist *et al*. 1993, Butler *et al*. 2008], although factors such as breed, geographical location and season are also influential [Ellis *et al*. 2006, Collomb *et al*. 2008, Strzałkowska *et al*. 2009a]. In areas where cows are housed during a significant part of the year, the composition of feed is one of the most important differences between organic and conventional dairy farming. Milk and dairy products from certified organic dairy production systems have been reported to contain higher concentrations of polyunsaturated fatty acids (PUFA), αLA (the main n-3 FA in milk), and/or CLA, and fat-soluble antioxidants than those from high-input conventional production systems [Jahreis *et al*. 1997, Bergamo *et al*. 2003, Ellis *et al*. 2006, Chilliard *et al.* 2007, Fall and Emanuelson 2011]. This is mainly due to an increased forage-to-concentrate ratio that increases milk PUFA and n-3 FA proportions and a decreased SFA proportion [Dewhurst *et al*. 2006] as well as feeding cows fresh herbage that enhances these trends, while also increasing milk *trans*-11 C18:1, *cis*-9, *trans*-11 conjugated linoleic acid (CLA), and C18:3n-3 proportions [Chilliard *et al*. 2007]. However, feeding regimes used in organic and conventional herds during the indoor feeding period are becoming increasingly similar when compared with the outdoor feeding period. In order to allow milk from organic production systems to be marketed as having 'added nutritional value' throughout the year, efforts need to be made to ensure higher concentrations of at least some nutritionally desirable compounds during the indoor feeding period [Butler *et al*. 2008]. This could be achieved by supplementation of conserved forage-based winter diet with oil seeds (e.g. rape seed, linseed, sunflower seed) that has been shown to significantly improve

concentrations of α-LA, VA, CLA and/or fat-soluble antioxidants in milk [Collomb *et al*. 2004, 2006, Lock and Bauman 2004, Dhiman *et al*. 2005, Shingfield *et al*. 2005, Chilliard *et al*. 2007].

The aim of the study was to evaluate the effect of the diet containing a supplemental mixture with alternative components suitable for the indoor feeding period of organic herds on the yield and composition of milk and the fatty acid profile.

Material and methods

Animals and diets

The on-farm experiment (a family farm, altitude 212 m above sea level; total annual rainfall 483-515 mm; mean annual temperature 9.7°C; 60 Czech Fleckvieh x Holstein cows, indoor feeding system based on maize silage, hay and a supplemental mixture) was carried out on twelve lactating cows (mean lactation 2.3±0.31, on average 116±29.5 days in milk) with similar milk production (26.3±1.66 kg/d) that were divided into 2 groups of six animals with similar milk yields. Cows were fed individually twice daily (5.00 and 15.00 h) *ad libitum* the diet based on maize silage, lucerne hay and a supplemental mixture (Tab. 1). The control group (C) was fed a control supplemental mixture; it is commonly fed in the herd, while the experimental group (E) was fed a supplemental mixture, in which soybean components and rapeseed extraction meal were replaced with rapeseed pomace and sunflower pomace as oil seed compounds. Furthermore, wheat germs were included into the E mixture to balance the CP content after the elimination of soybean components to ensure isonitrogenous and isoenergetic diets. The feeding components for the E groups were

Table 1. Composition of diets (kg/d, as fed basis) and composition of supplemental mixtures used in the experiment (g/kg)

Item	C	E
Maize silage (kg/d)	30	30
Lucerne hay (kg/d)	4	4
Supplemental mixture (kg/d)	8	8
Composition of supplemental mixtures		
wheat (g/kg)	250	100
barley (g/kg)	235	50
soybean extraction meal, peeled, toasted (g/kg)	212	
rapeseed extraction meal (g/kg)	170	
rapeseed pomace (g/kg)		250
sunflower pomace (g/kg)		120
wheat germ (g/kg)		200
malt sprouts (g/kg)	80	238
macrominerals (g/kg)	54	40
microminerals and vitamines (g/kg)	1	2

C – control diet based on soybean products. E – experimental diet based on rapeseed and sunflower products.

selected considering their local availability and suitability for organic farming.

Prior to the experiment there was at least a 1-week period to adapt to the type of diet. The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days. Each period consisted of a 7-d preliminary period and a 7-d experimental period.

Sampling and analyses

During the experiment the feed intake was monitored daily, feed samples were taken twice in each period for subsequent analyses. Dry matter (DM) was determined by drying at 55°C for 24 h, followed by milling through a 1 mm screen and drying for another 4 h at 103°C. Contents of crude protein (CP), crude fiber (CF), ash and fat were determined according to AOAC [1984]. Neutral detergent fiber (NDF, with α-amylase) was determined according to Van Soest et al. [1991], ash-free acid detergent fiber (ADF) was determined according to Goering and Van Soest [1970].

Cows were milked twice a day (6.00 and 16.00 h). Milk yield was recorded at each milking. During the experimental period, samples of milk were taken from each milking for the first three days. Samples from evening and morning milkings were mixed at a proportion corresponding to milk yields into one representative bulk sample per cow and day. Samples for the determination of basic milk constituents and somatic cell count were conserved with 2-bromo-2-nitropropane-1.3-diol (Bronopol; D&F Control Systems, Inc. USA) and cooled to 6°C. Milk samples for measurements of freezing point depression and electrical conductivity were treated only using low temperature, i.e. with no chemical preservation. Samples for the determination of the fatty acid (FA) profile were kept frozen at -20°C until subsequent analyses.

Contents of milk fat, crude protein, casein, lactose, solids–not–fat, urea and citric acid were measured using a Lactoscope FTIR apparatus (Delta Instruments, the Netherlands). Contents of crude protein and casein were calibrated by the reference Kjeldahl's method using the instrument line Tecator with a Kjeltec Auto Distillation unit 2 200 (Foss-Tecator AB, Sweden) according to the standard ČSN 57 0530. Urea was calibrated based on the spectrophotometric values at 420 nm wavelengths, respectively, using a Spekol 11 instrument (Carl Zeiss Jena, Germany). Content of citric acid was calibrated based on spectrophotometry at a 428 nm wavelength using Spekol 11 as described by Hanuš *et al*. [2009]. Acetone was determined by spectrophotometry at a 485 nm wavelength, respectively, also using Spekol 11. Milk freezing point depression (MFP) was analyzed using a top Cryo-Star automatic cryoscope by Funke-Gerber (Germany). Electric conductivity (EC) was measured using an OK 102/1 conductometer (Radelkis, Hungary) at 20°C (in $mS.cm^{-1}$). Active acidity (pH) was measured using the CyberScan 510 pH-meter (Eutech Instruments) at 20°C. Somatic cell count (SCC, in thousand/ml) was determined using a Fossomatic 90 instrument (Foss Electric, Denmark), which operated under calibration according to the direct microscopic results. Milk fat free fatty acid content (FFA, mmol/100 g) was determined using a Lactoscope FTIR apparatus (Delta Instruments, The Netherlands),

which operates on the infra-red spectroscopy principle with Fourier´s transformation and takes measurements under regular calibration according to the reference results (ČSN 57 0533) with specific modifications.

Fatty acids were determined by gas chromatography (GC) using a Varian 3800 apparatus (VARIAN TECHTRON, USA) with the following characteristics: a CP-Select CB FAME column, 50 m x 0.25 mm, 0.25 μm thickness programmed at 55°C for 5 min; 40°C/min up to 170°C; 2.0°C /min up to 196°C; 10.0°C /min up to 210°C. The temperature of both the injector and detector was 250°C. Helium at a flow rate of 1.8 ml/min was used as a carrier gas. Fatty acid methyl esters (FAME) were detected with a flame ionisation detector (FID). Milk fat was extracted with petroleum ether from freeze-dried milk samples. Fatty acids of isolated fat were re-esterified to their FAME by methanolic solution of potassium hydroxide. The identification of FAME was carried out using the analytical standards (SUPELCO, USA) and acetonitrile chemical ionization mass spectrometry (VARIAN MS 4000 detector). The proportions of individual FAs were calculated from the ratio of their peak area to the total area of all the observed FAs. In total, forty five FAs were identified.

Calculations and statistical analyses

For the calculation of indexes the following equations were used:

Atherogenic index [Ulbricht and Southgate 1991]:
AI = (C12 + 4 × C14 + C16)/ total UFAs
Desaturation index [Chilliard and Ferlay 2004]:
DI = C18:1n9c/(C18:0 + C18:1n9c)
Spreadability index of produced butter [Timmen 1990]:
SI = C18:1n9c/C16:0

Data obtained in the experiment were analysed using the GLM procedure of the Statistica 7.0 software package (StatSoft, Inc.), according to the following unitrait linear model:

$$y_{ijkl} = \mu + T_i + C_j + P_k + D_l + (T\,C)_{ij} + e_{ijkl}$$

where:

y_{ijkl} – ijkl-th observation;
μ – overall mean;
T_i – treatment (supplementation mixture) effect (i = 2);
C_j – cow effect (j = 12);
P_k – period effect (k = 2), D_l = day of sampling effect (l = 3);
TC_{ij} – interaction treatment by cow;
e_{ijkl} – residual connected with ijkl-th observation.

Results and discussion

During the indoor feeding period the feeding regimes are similar in conventional and organic farming systems because both systems use conserved forage-based diets. Butler *et al*. [2008] in their comparison between conventional and organic herds in the indoor period found only few significant differences and trends in milk fat composition. They suggested supplying conserved forage-based winter diets with oil seeds to maintain the desirable composition of milk fat, which was well documented in many studies [Lock and Bauman 2004, Collomb *et al*. 2004, 2006, Shingfield *et al*. 2005, Dhiman *et al*. 2005, Chilliard *et al*. 2007]. The alternative supplemental mixture used in our study contained rapeseed and sunflower pomace as main lipid supplements.

Nutrient intake and milk performance

The nutrient intake and milk response data are summarized in Tables 2 and 3, respectively. Daily intake of nutrients was calculated according to NRC [2001] and was comparable in the analyzed groups. Similar results were reported in other studies comparing diets containing soybean and rapeseed products [e.g. Kudrna and Marounek 2006, Veselý *et al*. 2009]. Milk production and contents of milk fat, protein, casein and solids-not-fat were not affected by the treatment ($P>0.05$). A similar finding was reported by Kudrna and Marounek [2006]. On the other hand, Givens *et al*. [2003] and Veselý *et al*. [2009] observed a decreased milk yield after feeding diets based on extruded rapeseed cake. Contents of lactose and urea were lower in C than in E ($P<0.05$). Since a close correlation exists between the rumen-degraded protein

Table 2. Average daily nutrient intake (according to NRC, 2001)

Item	C	E
Dry matter (kg/d)	20.4	20.4
Ash (kg/d)	1.77	1.62
NDF[1] (kg/d)	6.61	7.25
Fat (kg/d)	0.52	0.91
Crude protein (kg/d)	3.46	3.52
RDP[2] (kg/d)	2.26	2.29
RUP[3] (kg/d)	1.20	1.23
NEL[4] (MJ/d)	133	131
MP[5] (kg/d)	1.99	1.79

C – control diet based on soybean products. E – experimental diet based on rapeseed and sunflower products.
[1] Neutral detergent fiber.
[2] Rumen degradable protein.
[3] Rumen undegradable protein.
[4] Net energy for lactation.
[5] Metabolizable protein.

Table 3. Yield and composition of milk in the control (C) and experimental (E) group of cows

Item	C	E	SEM	P-value
Milk yield (kg/d)	23.87	23.92	0.163	0.8387
Fat (g/kg)	41.5	41.6	0.055	0.8725
Protein (g/kg)	31.9	31.8	0.010	0.5827
Lactose (g/kg)	47.3	47.7	0.009	0.0081
Solids-not-fat (g/kg)	85.2	85.6	0.017	0.1125
Casein (g/kg)	25.0	24.8	0.013	0.1483
Urea (mg/100 ml)	24.4	32.6	0.414	<0.001
Milk freezing point (°C)	-0.5311	-0.5255	0.001	0.0015
Electric conductivity (mS/cm)	4.24	4.15	0.031	0.0456
Free fatty acids (mmol/100 g)	0.68	0.65	0.018	0.2601
Citric acid (mmol/l)	8.23	8.25	0.059	0.8675
Acetone (mg/l)	3.01	3.27	0.289	0.5327
pH ( )	7.04	7.01	0.003	<0.001

C – control diet based on soybean products. E – experimental diet based on rapeseed and sunflower products. SEM – standard error of the mean.

balance in the ration and urea concentration in milk [Schepers and Meijer 1998], differences between milk urea content found in our study may be explained by different degradability of oilseed feeding components used as suggested by Kudrna and Marounek [2006]. When minor milk constituents are concerned, contents of FFAs, citric acid and acetone did not differ significantly between treatments ($P>0.05$). Milk freezing point was higher in C than in E ($P<0.05$). Electric conductivity and pH were higher in C in comparison to E ($P<0.05$). Differences in milk freezing point determined in our experiment are associated with different contents of lactose and urea that have been identified, among others, as biotic factors influencing milk freezing point depression [Hanuš *et al*. 2011b].

Milk fatty acid profile

A total of 45 FA were identified applying the described analytical method used in FA determination. Some determined isomers were not specified (see Tab. 4 with comments in footnotes).

Saturated fatty acids

The dietary effect on milk FA profiles is presented in Table 4 and sums of FA depending on their chain length and saturation are given in Table 5. The total SFA content was higher in C in comparison to E ($P<0.05$). After the inclusion of alternative products (rapeseed and sunflower pomace) into the E diet the contents of C4:0, C18:0, C20:0 and C22:0 increased, while contents of C6:0, C8:0, C10:0, C12:0, C14:0, C16:0 and C17:0 decreased in comparison to the control diet (C) containing soybean products ($P<0.05$). An increased content of C4:0 was observed also by Glasser *et al.* [2008] after feeding rapeseed oils, soybean seeds, and protected

Table 4. Milk fatty acid profile (%)

Fatty acid	C	E	SEM	P-value
C4:0	1.528	1.663	0.035	0.009
C6:0	1.567	1.564	0.019	0.921
C8:0	1.262	1.208	0.013	0.005
C10:0	3.064	2.722	0.040	<0.001
C10:1[1]	0.288	0.249	0.005	<0.001
C11:0	0.064	0.047	0.003	<0.001
C12:0	3.826	3.236	0.051	<0.001
C12:1[1]	0.088	0.055	0.003	<0.001
C12:1+isoC13:0	0.116	0.098	0.003	<0.001
C13:0	0.113	0.092	0.003	<0.001
isoC14:0	0.088	0.084	0.001	0.101
C14:0	11.990	10.378	0.090	<0.001
C14:1n5cis+isoC15:0	1.146	0.946	0.016	<0.001
anteisoC15:0	0.460	0.458	0.004	0.686
C15:0	1.108	0.954	0.011	<0.001
isoC16:0	0.265	0.245	0.003	<0.001
C16:0	34.182	28.013	0.168	<0.001
C16:1[1]	0.159	0.157	0.002	0.486
C16:1n7cis	1.641	1.221	0.026	<0.001
isoC17:0	0.439	0.473	0.004	<0.001
C16:1[2]	0.200	0.145	0.004	<0.001
anteisoC17:0	0.514	0.494	0.007	0.046
C17:0	0.566	0.505	0.006	<0.001
C17:1n7cis	0.255	0.231	0.004	<0.001
C18:0	8.362	11.649	0.159	<0.001
C18:1t [3]	0.418	0.796	0.013	<0.001
C18:1n7t	1.152	2.374	0.026	<0.001
C18:1n9cis	18.753	22.085	0.195	<0.001
C18:1n7cis	1.163	1.197	0.013	0.065
C18:1[2]	0.498	0.763	0.004	<0.001
C18:1[2]	0.400	0.600	0.005	<0.001
C18:1+C18:2	0.327	0.505	0.004	<0.001
C18:2[1]	0.176	0.252	0.004	<0.001
C18:2n6cis,cis	2.413	2.745	0.022	<0.001
C19:1	0.131	0.133	0.004	0.724
C18:3[1]	0.038	0.048	0.002	<0.001
C18:3n3cis	0.227	0.252	0.002	<0.001
C20:0	0.122	0.168	0.002	<0.001
C18:2 CLA 9,11[4]	0.407	0.691	0.009	<0.001
C20:1n9cis	0.109	0.143	0.002	<0.001
C20:3n6cis	0.090	0.089	0.002	0.613
C20:4n6cis	0.163	0.138	0.002	<0.001
C22:0	0.025	0.041	0.002	<0.001
C24:0	0.062	0.061	0.001	0.526
C22:5n3cis	0.034	0.033	0.004	0.818
Total	100	100		

C – control diet based on soybean products. E – experimental diet based on rapeseed and sunflower products. SEM – standard error of the mean.
[1] Unspecified isomers.
[2] Isomer n<7cis.
[3] Trans isomers.
[4] Cis, trans (trans, cis)-9,11-octadecadienic acid.

Table 5. Total fatty acids depending on saturation and chain length and indexes characterising technological properties of milk fat

Item	C	E	SEM	P-value
SFA	69.64	64.09	0.21	<0.001
UFA	30.39	35.95	0.21	<0.001
MUFA	26.52	31.19	0.21	<0.001
PUFA	3.88	4.75	0.03	<0.001
SFA/UFA	2.35	1.84	0.02	<0.001
SFA/MUFA	2.70	2.12	0.03	<0.001
SFA/PUFA	18.21	13.93	0.15	<0.001
MUFA/PUFA	6.86	6.60	0.07	0.017
Short-chain FA[1]	11.69	10.74	0.13	<0.001
Medium-chain FA[2]	53.24	44.49	0.25	<0.001
Short+medium-chain FA	64.93	55.24	0.35	<0.001
Long-chain FA[3]	35.07	44.76	0.35	<0.001
C12:0+C14:0+C16:0	50.35	41.96	0.27	<0.001
PUFA n-3 series	0.67	0.98	0.01	<0.001
PUFA n-6 series	2.70	3.02	0.03	<0.001
PUFA n-6/PUFA n-3	4.18	3.17	0.05	<0.001
AI[4]	1.706	1.214	0.019	<0.001
DI[5]	0.690	0.655	0.003	<0.001
SI[6]	0.565	0.826	0.009	<0.001

C – control diet based on soybean products. E – experimental diet based on rapeseed and sunflower products. SEM – standard error of the mean. SFA – saturated fatty acids. UFA – unsaturated fatty acids. MUFA – monounsaturated fatty acids. PUFA – polyunsaturated fatty acids.
[1] Fatty acids of carbon chain length C4-C12.
[2] Fatty acids of carbon chain length C14-C16.
[3] Fatty acids of carbon chain length of C18 and more.
[4] Atherogenic index.
[5] Desaturation index.
[6] Spreadability index.

sunflower seed. Similar findings were reported by e.g. Focant *et al*. [1994], Komprda *et al*. [2000], Kudrna and Marounek [2006] and Veselý *et al*. [2009], who performed their studies on rapeseed dietary components. Although a high proportion of SFAs was found in milk from conserved forage and concentrate-rich diets [Dewhurst *et al*. 2006], according to Coppa *et al.* [2013] the concentration of *de novo* synthesized FAs (C4:0-C14:0) and C16:0 decreased with with an increase in fresh herbage and grass silage proportions in cow diet. This is in accordance with our findings suggesting that alternative components in the supplemental E mixture had a similar effect to that of inclusion of fresh herbage to the diet. Furthermore, increasing the fresh herbage proportion in the diet also increased PUFA intake, which is known to partially inhibit *de novo* FA synthesis [Elgersma *et al*. 2006]. In our study, the alternative feeding mixture had a similar effect, because PUFA content in E increased significantly ($P<0.001$). Among the long-chain SFAs, C18:0 was strongly affected by the treatment ($P<0.001$). According to Coppa *et al*. [2013], C18:0 is the final product of dietary

PUFA biohydrogenation and may be affected by plant secondary metabolites, but also by the type of concentrates as summarised by Glasser *et al*. [2008].

Unsaturated fatty acids

In our study, the total content of UFA, MUFA, PUFA and PUFA n-3 and n-6 series was higher in E than in C (P<0.001). Similarly, Komprda *et al*. [2000] reported significant increases in total PUFA and PUFA n-6 series when feeding a diet with heat-treated rapeseed cake in comparison with the soybean meal diet, while the concentration of PUFA n-3 was not affected by the treatment. On the other hand, Kudrna and Marounek [2006] and Veselý *et al*. [2009] reported a more pronounced positive effect of feeding extruded soybeans on the above-mentioned values than that of feeding rapeseed cake. These differences have probably arisen from differences in the composition of the diets (various portions of soybean or rapeseed components).

The concentration of oleic acid (C18:1n9c) in our study was considerably higher in E than in C (P<0.001). These results are in agreement with those of Kudrna and Marounek [2006] and Veselý *et al*. [2009], who reported a tendency to increased levels of oleic acid in milk when feeding the rapeseed cake diet compared to feeding the extruded soybean diet. According to Focant *et al*. [1994], Bayourthe *et al*. [2000], Collomb *et al*. [2004] and Dang Van *et al*. [2011] inclusion of rape seeds in the diet increased the concentration of long-chain FAs (mainly C18:0, C18:1, and C18:2) and oleic acid, while it decreased concentrations of short and medium chain FAs in milk.

The C18:2n6 is considered as an indicator of maize silage-based diets, as maize is rich in this FA [Ferlay *et al*. 2008, Slots *et al*. 2009]. However, soybean and sunflower supplements are also important sources of C18:2n6, which increase its proportions in milk [Chilliard *et al*. 2007, Glasser *et al*. 2008], even in fresh herbage- or conserved herbage-based diets. Similarly, in our experiment the inclusion of sunflower pomace into the E diet resulted in an increased content of C18:2n6 (P<0.001) in the maize silage-based diet.

The concentration of CLA *cis9, trans11* was higher in E than in C (P<0.001). While comparing production systems, Butler *et al*. [2008] found that concentrations of CLA *cis9, trans11* were significantly higher in milk from low input rather than high input systems, and they suggested that it was likely to be caused by the contrasting effects of applied diets in the above mentioned systems on the biosynthesis of this CLA isomer. According to Glasser *et al.* [2008], total CLA and CLA *cis9, trans11* were significantly increased by all lipid supplements (rape seeds, linseed, sunflower seed and soybean in the form of seeds, protected seeds or oils), except for rape in the form of both as seeds and oils.

According to Glasser *et al*. [2008], percentages of FAs with 20 carbons or more are generally weakly affected by lipid supplements applied in the form of oil seeds or protected oil seeds. Similar results were observed in our study where only concentrations of C20:0, C20:1, C20:4 and C22:0 differed significantly between groups; however, their concentrations were low.

PUFA n6:n3 ratio

According to Simopoulos [2002], the high ratio of total n-6 to n-3 FAs in the present-day human diets has been highlighted in recent years as a promoter of the pathogenesis of cardiovascular disease, cancer, inflammatory and autoimmune diseases, whereas increased levels of total n-3 FAs (a low total n-6 to n-3 ratio) exert suppressive effects. From this point of view the ideal ratio is 1:1 [Simopoulos 2002]. Results of many recent studies demonstrated that the organic management system yielded higher total n-3 and n-6 fatty acids and a lower n-6 : n-3 ratio [Ellis *et al*. 2006, Lavrenčič *et al*. 2007, Bloksma *et al*. 2008, Butler *et al*. 2008, Collomb *et al*. 2008, Slots *et al*. 2009, Fall and Emanuelson 2011]. In our study, E milk had a ratio of 3.17:1 when compared with the C milk with a ratio of 4.18:1. Both values are considerably higher than those reported in the above mentioned studies, ranging from 1.3:1 to 2:1 in organic milk and from 2.2 to 4.7:1 in conventionally produced milk. According to those studies, the main reason for the differences was found in the amounts of grass and grass silage compared with the amounts of concentrates given to the cows. Furthermore, a seasonal effect on this ratio has also been documented [Ellis *et al*. 2006].

Indexes

Indexes characterising technological and health-promoting properties of milk are calculated in Table 5. The nutritional and physical properties of milk and dairy products are influenced by the length of the carbon chain in FAs, their degree of (un)saturation and their positional distribution within the triacylgycerol molecules [Hillbrick and Augustin 2002]. For example, the presence of longer-chain SFAs increases butter hardness, while milk with a high proportion of UFAs (typical range of 275-400 g/kg fat) tends to give softer products [e.g. more spreadable butter, Chen *et al*. 2004]. Unsaturated (especially polyunsaturated) FAs are also more prone to oxidation, which results in the development of off-flavour and reduced shelf-life in milk and dairy products [Chen *et al*. 2004]. Furthermore, in the group of SFAs, lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0) were found to be detrimental from the point of view of cardiovascular disease control [Jensen 2002]. It was demonstrated that a higher consumption of these FAs increases concentrations of low-density lipoprotein, whereas a greater consumption of UFAs has the reverse effect [e.g. Fernandez and West 2005]. These aspects, i.e. contents of lauric, myristic, palmitic and unsaturated acids in milk fat are taken into account in the atherogenic index (AI) that is used as a risk indicator for cardiovascular diseases [Ulbricht and Southgate 1991]. In our study, the sum of the above mentioned SFAs was higher in C in comparison to E, amounting to 50.00 and 41.63% ($P < 0.001$), respectively, suggesting a more favourable composition of E milk. Proportions of these FAs in both groups are in accordance with findings of Pešek *et al.* [2006] in the Czech Fleckvieh breed population (mean - 45.26%, min - 35.39 and max - 54.31%). Similarly, AI calculated in our study was lower in E than in C ($P<0.05$). Similar tendencies (although non-significant) were also reported by Veselý

et al. [2009]. On the other hand, Kudrna and Marounek [2006] described a tendency to a decreased AI in the extruded soybean diet in comparison with the rapeseed cake diet. This discrepancy is probably caused by differences in concentrations of C12:0 and C16:0. The spreadability index (SI) calculated in our experiment was higher in E than in C ($P < 0.05$), which is in agreement with the findings of McNamee *et al*. [2002], who described that rapeseed-based diets resulted in an increased ratio of C18:1n9c/C16:0 and therefore produced softer butter fat. Kudrna and Marounek [2006] and Veselý *et al.* [2009] found only tendencies to increased SI after inclusion of rapeseed products into the diet. However, it should be taken into account that our E diet apart from rapeseed contained also sunflower components, which also contributed to changes in the FA profile and the above mentioned indexes.

In conclusion, rapeseed and sunflower products have become dietary components frequently used in dairy cow nutrition, suitable also for organic agriculture as an adequate substitution of soybean products. A direct comparison of the diet fed in the conventional herd with the proposed alternative diet suitable for organic herds resulted in a more favourable fatty acid profile, i.e. a higher proportion of unsaturated and long chain FAs at the expense of saturated and short- and medium-chain FAs, as well as a lower ratio of total n-6 to n-3 fatty acids of experimental milk without a detrimental effect on milk yield or composition.

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