

Milk fatty acid profile in cows as influenced by diet supplementation with rapeseed pomace and extruded full-fat soya in different feeding periods

Kristina Musayeva¹, Antanas Sederevičius¹, Ingrida Monkevičienė¹, Judita Žymantienė¹, Vaidas Oberauskas¹, Sigita Kerzienė², Aldona Baltušnikienė³, Janina Černauskienė⁴, Rasa Želvytė¹

Lithuanian University of Health Sciences,

¹Veterinary Academy, Veterinary Faculty, Department of Anatomy and Physiology,

²Veterinary Academy, Faculty of Animal Science, Department of Animal Breeding,

³Medical Academy, Medicine Faculty, Department of Biochemistry,

⁴Veterinary Academy, Faculty of Animal Science, Department of Animal Nutrition, Kaunas, Lithuania

Received June 18, 2020

Accepted February 24, 2021

Abstract

The aim of the study was to estimate the effect of rapeseed pomace and extruded full-fat soya in the diets of dairy cows on changes in the milk fatty acid (FA) profile. The experiment was carried out on 30 dairy cows and divided into two periods: the indoor feeding period and the grazing period. Control diet cows were fed the farm's total mixed ration (TMR); cows of experimental group 1 (ES) cows were fed the farm's TMR supplemented with extruded full-fat soya; cows of experimental group 2 (RP) were fed the farm's TMR supplemented with rapeseed pomace. The total content of saturated fatty acids (SFAs) tended to be lower in the RP and ES groups than in the control group during both feeding periods. Our results suggest that supplementing diets with rapeseed and soybean products are both effective in improving the fatty acid proportion of desirable (hypocholesterolaemic) FAs, mainly oleic acid (18:1n-9), linoleic acid (18:2n-6) and conjugated linoleic acid (CLAcis9trans11). The study showed that during the indoor feeding period, the unsaturated fatty acid (UFA) contents were significantly higher in the ES group compared to the control group ($P < 0.05$). The nutritional value of milk from the feeding groups in which rations were supplemented with ES or RP was better due to a quartile reduction of the atherogenicity index (AI) and the significantly increased spreadability index (SI) of the butter manufactured during the indoor feeding period ($P < 0.01$).

Milk fat quality, dietary supplements, health

In recent years, an increase in the consumption of raw milk has been noted, accompanied by the assumption that raw milk gives higher health benefits, nutrition values and potential probiotic bacteria (Claeys et al. 2014). Bovine milk lipids have been characterized as a very high digestibility fat. The human body assimilates 97–99% of milk fat. In the human body, short-chain fatty acids (SCFA) are used as sources of energy for the muscles, heart, liver, kidneys, blood platelets, and nervous system. *In vitro* and *in vivo* studies have demonstrated a wide range of health benefits of omega-6 and (in particular) omega-3 polyunsaturated fatty acids (PUFAs), such as lowering the risk of cardiovascular diseases, type 2 diabetes, hypertension, cancer, and specific neurological dysfunctions (Micinski et al. 2012).

Ruminant milkfat is of unique composition among terrestrial mammals, due to its great diversity of component fatty acids (FAs). The diversity arises from the effects of ruminal biohydrogenation on dietary unsaturated FAs (UFAs) and the range of FAs synthesized *de novo* in the mammary gland (Markiewicz-Keszycka et al. 2013). The UFAs are usually called 'healthy fats', especially for their impact on the level of cholesterol in blood

Address for correspondence:

Kristina Musayeva
Lithuanian University of Health
Sciences Veterinary Academy
Tilžės g. 18, LT-47181, Kaunas, Lithuania

Phone: +370 37 36 23 83
E-mail: kristina.musayeva@lsmuni.lt
<http://actavet.vfu.cz/>

(Haug et al. 2007; Arnould and Soyeurt 2009). Milk contains a low concentration of beneficial UFAs, including conjugated linoleic acid (18:2cis9trans11), α -linolenic (ALA; 18:3n-3) and oleic (OA; 18:1cis9) acids, which could be improved in milk through pasture feeding (Simopoulos 2002; Nantapo et al. 2014).

Many factors are associated with the variations in the amount and FAs composition of bovine milk lipids. They may be of animal origin, including the genetics (breed and selection), stage of lactation, mastitis, and ruminal fermentation, or they may be feed-related factors, which comprise fibre and energy intake, dietary fats, and seasonal and regional effects (Lindmark-Månsson et al. 2003).

The FA composition of cows' milk became less favourable to human health in the last decades due to changes in feeding and management practices with notably higher proportions of concentrates and silages in diets and less grazing (Elgersma et al. 2006). When compared with the total mixed ration (TMR) fed, grazing dairy cows produced milk fat with higher concentrations of monounsaturated FAs (MUFAs), OA and vaccenic acid (VA), PUFAs and conjugated linoleic acids (CLA), and lower concentrations of hypercholesterolaemic FAs (12:0, 14:0, and 16:0) (Chilliard et al. 2007; Elgersma 2015).

Thus, there is heightened interest in the manipulation of the FA profile of milk fat by lowering the saturated fatty acid (SFA) concentration and increasing the UFA concentration (Vesely et al. 2009). By modifying diets of lactating cows, MUFA content can be increased by 50 to 80% and may approach 50% of milk FAs by feeding lipids rich in 18-carbon FAs (Chouinard et al. 1998). Feeding low roughage diets increases the proportion of OA in milk fat, and the palmitic acid (PA, 16:0) content in milk fat can be reduced by 20 to 40% (Gulati et al. 1997; Demeyer and Doreau 1999).

In recent years, rapeseed in the form of meal or cakes with varying fat contents has become a common component of dairy cow diets. Soya bean is frequently fed to dairy cows as well. The lipids of rapeseed and soya bean are highly unsaturated with OA and linoleic acid (LA) as their principal components, respectively (McNamee et al. 2002). The inclusion of rapeseed products to the diet of dairy cows changes the milk FA profile because lipids of rapeseed are highly UFAs, with OA and LA as the principal components (Chouinard et al. 1997; McNamee et al. 2002). According to Focant et al. (1994), extruded rapeseeds in the diet increase the concentration of long-chain FAs (mainly 18:0, 18:1 and 18:2) in milk and decrease the concentration of PA. Heat-treated full-fat soybean presents an interesting FA profile that can improve the quality of fat in animal products according to consumer demand of healthier foods (Chilliard et al. 2000).

The purpose of the present experiment was to estimate the effect of rapeseed pomace and extruded full-fat soya in the diets of dairy cows on changes in the milk FA profile during the grazing and indoor feeding periods. Several indexes summarizing various effects of different FAs were calculated.

Materials and Methods

All procedures were carried out according to the animal usage protocol approved by the National Institutional Experimental Animal Ethical Commission, which adheres to the requirements laid down in Directive 2010/63/EEC. All experiments were approved by the State Food and Veterinary Service by official letter No. G2-60 of 15 February 2017.

Experimental design and animal feeding management

The experiment was conducted on a dairy farm at the Practical Training and Trial Centre of the Veterinary Academy at the Lithuanian University of Health Sciences. Cows were housed in a cowshed and had free stalls. The experiment was performed during the indoor feeding period (from 1 November to 26 December) and the grazing period (from 22 May to 16 July). Each of these experimental periods were divided in two: the first three weeks of the trial were used as an adaptation period, while during the 56 days milk samples were collected daily

during both the indoor feeding and grazing period. The cows were milked twice a day at 05.00 h and 17.00 h in a milking parlour.

At the beginning of the experiment, 30 Holstein cows were selected on the basis of milk yield (35.0 ± 5.7 kg/d during grazing and 30.05 ± 1.7 kg/d during the indoor feeding period), the days of lactation (80 ± 41 d), body weight (650 kg on average) and parity (2.3 ± 1.4). Cows were divided into three groups (10 cows per group) according to feeding: the control group cows were fed the farm's total mixed ration; cows of experimental group 1 (ES) were fed the farm's total mixed ration with extruded full-fat soya; and cows of experimental group 2 (RP) were fed the farm's total mixed ration with rapeseed pomace. Cows of all groups were fed *ad libitum* and milked individually using automated milking systems. The composition of the diets is presented in Table 1.

Table 1. The composition and nutritive value of cow's diets during different feeding periods.

Feedstuffs	Indoor feeding period			Grazing period		
	Control	ES	RP	Control	ES	RP
Pasture grass, kg	-	-	-	80	80	80
Maize silage, kg	8.0	8.0	8.0	10.0	10.0	10.0
Wheat straw, kg	2.0	2.0	2.0	2.0	2.0	2.0
Perennial grass haylage, kg	25.0	25.0	25.0	6.0	6.0	6.0
Compound concentrates for milking cows, kg	6.1	4.1	4.6	-	-	-
Barley flour, kg	-	-	-	6.0	4.8	4.5
Poaceae and legumes grass hay, kg	-	-	-	2.0	2.0	2.0
Fodder sugar, kg	0.2	0.2	0.2	0.2	0.2	0.2
Rapeseed pomace, kg	-	-	1.5	-	-	1.5
Soya bean (extruded), kg	-	1.5	-	-	1.2	-
Premix Provimi, g	200	200	200	-	-	-
Cow mineral supplement, g	-	-	-	200	200	200
NaCl, g	80	80	80	100	100	100
Chalk, g	150	150	150	150	150	150
Each kg of dry matter in the diet contains:						
Net energy for lactation (NEL), MJ	6.48	6.52	6.49	6.32	6.45	6.37
Crude protein, %	16.9	18.2	17.9	16.5	18.7	18.1
Crude fat, %	3.35	4.01	3.71	3.10	3.90	3.42
Crude fibre, %	22.30	22.56	22.80	20.52	20.47	21.79
Calcium ratio with phosphorus	1.73:1	1.70:1	1.75:1	1.88:1	1.85:1	1.67:1

ES - total mixed ration with extruded full-fat soya; RP - total mixed ration with rapeseed pomace

The grazing period began in May. Cows of each group (control and treatment) were grazed in individual fenced areas of the same meadow. During the grazing period, the diet consisted mainly of grazing grass, maize silage, hay and additives: salt, cow mineral supplement, chalk. The dairy cows grazed on a nutritionally rich pasture for two weeks, and after the adaptation period of 21 days, experimental controls were performed. Pasture sward was composed of 72.0% grasses (*Ranunculus acris*, *Poa pratensis* L., *Festuca pratensis* Huds, *Phleum pratense* L., *Dactylis glomerata* L.), 11.9% legumes (*Trifolium repens* L., *Trifolium hybridum* L., *Lotus corniculatus* L.) and 8.1% Fabaceae (*Trifolium repens*, *Trifolium pratense*). The supplement was offered to animals twice daily in the milking parlour (Table 2). A temporary electric fence was used to adjust the area to assure herbage allowance. Drinking water was always available on pasture.

Sampling procedure and laboratory analyses of feed

During the experiment, feed intake and efficiency were monitored and analysed. The dry matter intake (DMI) was calculated based on the dry matter (DM) weight of total mixed ration offered and feedorts. The feed efficiency was calculated based on the milk yield (kg per day) and feed intake (kg per day).

Feed samples were analysed for the concentration of DM, crude protein (CP), crude fibre, crude fat and crude ash (Faithfull 2002). Samples of all diet ingredients were taken and analysed in accordance with European Commission Regulation No. 691/2013 amending Regulation (EC) No.152/2009. The analyses were carried out at the Analytical Laboratory of the Institute of Animal Science, Lithuanian University of Health Sciences. Collected feed samples and three subsamples of each batch for chemical analyses were dried at 60 °C for 20 h and ground in an ultracentrifugal mill ZM 100 (Retsch GmbH, Germany) with a 1.0 mm sieve.

Table 2. The chemical composition and fatty acid (FA) profile (% of total FAs or mg/100 mg) of the extruded full-fat soya and rapeseed pomace supplements.

Chemical composition	ES	RP
Dry matter, %	89.3	89.8
Crude protein, % DM	36.1	33.6
Fat, % DM	18.6	15.9
Ash, % DM	5.4	5.6
Crude fibre, % DM	6.9	7.1
NEL (Net energy for lactation), MJ/kg	20.8	19.7
Fatty acid profile		
Palmitic acid, 16:0	24.13	23.05
Palmitoleic acid, 16:1	0.08	0.63
Stearic acid, 18:0	4.40	4.31
Oleic acid, 18:1n-9	20.28	25.31
Vaccenic acid, 18:1n-7	1.55	1.03
Linoleic acid, 18:2n-6	35.50	31.03
α -linolenic acid, 18:3n-3	7.18	5.68
Arachidic acid, 20:0	0.91	1.03
Eicosenoic acid, 20:1n-9	0.14	0.97
Behenic acid, 22:0	0.18	2.37
Lignoceric acid, 24:0	1.0	1.01
Other acids	4.44	4.94
Total SFA	35.38	34.29
Total UFA	64.62	65.71

DM – dry matter; ES – extruded full-fat soya; RP – rapeseed pomace; SFA – saturated fatty acids; UFA – unsaturated fatty acids

microcentrifuge (Eppendorf Centrifuge 5418). The concentrated fat was collected and directed for FAME preparation (Feng et al. 2004).

Extruded full-fat soya and rapeseed pomace supplements were also analysed for their fatty acids profile. The samples were previously ground through a 1 mm screen. Fatty acid methyl esters of feed lipids were prepared by a one-step extraction transesterification procedure using toluene, according to Sukhija and Palmquist (1988).

Sampling procedure and laboratory analyses of the fatty acid profile in milk

During the experimental period, samples of milk (100 ml) were taken from milk collected in the evening. Milk samples were collected daily over 35 days during both the indoor feeding and grazing periods. The samples for the determination of the FA profile were kept frozen at -20 °C until subsequent analyses. The FA profile was determined as follows (Standard GOST 31665-2012): concentrated fat (50 mg) was mixed with 4 ml of hexane (Sigma-Aldrich, Darmstadt, Germany) and 200 μ l of 2 mol-l KOH in methanol (Sigma-Aldrich, Darmstadt, Germany), intensively vortexed for 1 min, and, after 10 min of standing, the top layer was collected and filtered into a chromatography vial (Sigma-Aldrich, Darmstadt, Germany). FAs were released in the form of fatty acid methyl esters (FAMES), which were separated using a Shimadzu gas chromatograph GC-17A (Perkin-Elmer, Kyoto, Japan) equipped with a flame ionization detector (FID) and a BPX-70 capillary column 120 m \times 0.25 mm \times 0.25 μ m (Alltech associates, inc., USA). Conditions for chromatographic analysis were as follows: the injector and detector temperatures were 260 °C and 270 °C, respectively. Nitrogen was used as a carrier gas with a flow of 3 ml/min. The column was held at 60 °C for 3 min after an injection of 0.2 μ l, and the temperature was programmed at 20 °C/min to 220 °C. Then, the column was held at 250 °C for 40 min. The FAMES were detected with the FID, and the chromatographic peaks were identified on the basis of comparison with retention times of a mixture of reference material products made of raw milk for the analysis of fatty acids with gas chromatograph (GC). The certified shock frozen long-term standard (LZS) from QSE GmbH. Product No. 8500 FSM (EN ISO 5508:1990) was obtained from Sigma-Aldrich (Darmstadt, Germany). The proportions of individual FAs were calculated from the ratio of their peak area to the total area of all observed FAs peaks. The chemical composition and FA profile of extruded full-fat soya and rapeseed pomace ingredients are given in Table 2.

The CP content was determined by the Kjeldahl method, and conversion factors of 5.7 for wheat, 5.71 for soybean, and 6.25 for other ingredients were used to convert total nitrogen to crude protein (Van Soest et al. 1991). Crude fat was calculated by extracting it with petroleum ether using Soxtherm extraction system (Gerhardt GMBH & Co., Germany). Crude ash was calculated by burning the sample in a muffle furnace (CHOJI-1,6,2,5,1/11-ИЗ, Lithuania) at 550 °C for 3 h (Commission Regulation 2013). Crude fibre was determined as the residue after sequential treatment with hot sulphuric acid (H₂SO₄) 1.25% and hot sodium hydroxide (NaOH) 1.25% according to the Weende method with a Fibertec 2023 FiberCap system (Foss Tecator AB, Sweden). The samples were subjected to fibre component analyses with an ANKOM 220 Fiber Analyzer (ANKOM Technology, USA): acid detergent fibre (ADF) and neutral detergent fibre (NDF) and acid detergent lignin (ADL) using a cell wall detergent fractionation method according to Faithfull (2002).

Butter was manufactured according to standard methods (Walstra et al. 1999). Lipid extraction: the butter sample was melt in a warm water bath, mixed, weighed to 60 mg, and directed for FAME preparation. Three hundred and twenty ml of buttermilk sample were poured into 50 ml conical tubes and centrifuged for 30 min at 12,000 rpm at 4 °C (Thermo Scientific, Heraeus Multifuge X1R Centrifuge). The settled fat layer at the top of the tube was collected and transferred to 1.5 ml tubes (Eppendorf) for further fat separation (20 min 13,000 rpm, at 20 °C) by

Calculations and statistical analyses

For the calculation of indexes, the following equations were used:

Index of atherogenicity (Ulbricht and Southgate 1991):

$$AI = (LA + 4 \times MA + PA) / \text{sum of UFAs},$$

where lauric acid (LA, 12:0), myristic acid, (MA, 14:0), palmitic acid (PA, 16:0).

Index of spreadability of manufactured butter (Timmen 1990):

$$SI = OA/PA,$$

where oleic acid (OA; 18:1n-9) and palmitic acid (PA; 6:0).

Desirable (hypocholesterolaemic) fatty acids (DFAs) were expressed as the sum of UFAs and stearic acid (SA, 18:0) according to this formula (Medeiros et al. 2014):

$$DFAs = UFAs + SA$$

Statistical analysis was performed with the SPSS statistical package (version 20.0, SPSS Inc., Chicago, IL). The data were analysed using descriptive statistics (explore) and analysis of variance methods. For all statistical evaluations, the means and standard error of the mean (SEM) were used. In the case of a significant difference ($P < 0.05$) groups were compared by Tukey's test.

Results and Discussion

The dietary effect on milk FA profiles is presented in Table 3. During the indoor feeding period, the UFA content was significantly higher in the ES group compared to the control group ($P < 0.05$). After the inclusion of rapeseed pomace or extruded full-fat soya into the experimental groups diets, the contents of capric acid (CA, 10:0) were lower during grazing period in comparison to those in the control diet ($P < 0.01$).

During the grazing period, the amount of PA in milk varied significantly between the experimental groups ($P < 0.05$). While during the indoor feeding period, PA was lower in the ES group compared to the control group ($P < 0.01$). An increased content of butyric acid 4:0, stearic acid (SA, 18:0), OA, and LA and a decrease of CA, lauric 12:0, myristic 14:0, PA, palmitoleic 16:1 and heptadecanoic 17:0 acids were observed by Glasser et al. (2008) after feeding rapeseed oil, soya bean seeds, and protected sunflower seeds to cows. Similar findings were reported by Komprda et al. (2000), Kudrna and Marounek (2006) and Vesely et al. (2009), who performed their studies on rapeseed dietary components. Some researchers have determined that the concentrations of *de novo*-synthesized FAs 4:0-14:0 and PA were lower with an increase in fresh herbage and grass silage proportions in the cow's diet (Coppa et al. 2013). This is in accordance with our findings, suggesting that alternative components in the experimental group diets had a similar effect to that of the inclusion of fresh herbage to the diet.

During the grazing period, the SA content was strongly affected by the feeding ($P < 0.01$). According to Coppa et al. (2013), SA is the final product of dietary PUFA biohydrogenation and may be affected by plant secondary metabolites but also by the type of concentrates, as summarized by Glasser et al. (2008).

Among the MUFAs, OA was at a higher concentration in the experimental groups and was significantly affected by feeding ($P < 0.05$) during the indoor feeding period. These results are in agreement with those of Kudrna and Marounek (2006) and Vesely et al. (2009) who reported a tendency for higher concentrations of OA in milk when feeding cows with a rapeseed cake diet compared to feeding them with an extruded soya bean diet (Krizova et al. 2016).

Among the PUFAs, LA is considered an indicator of maize silage-based diets, as maize is rich in this FAs (Slots et al. 2009; Krizova et al. 2016). However, soya bean supplements are also important sources of LA which increases its proportions in milk (Glasser et al. 2008), even in fresh herbage- or conserved herbage-based diets. Similarly, in our experiment, the inclusion of rapeseed pomace and extruded full-fat soya into the experimental group diets resulted in a significantly ($P < 0.01$) higher content of LA in the maize silage-based diet (grazing period).

Table 3. The fatty acid composition of milk fat (% of total FAs) and calculated indexes during the grazing and indoor feeding periods in the different feeding groups.

FAs and indexes	Indoor feeding period					Grazing period				
	Control n = 45	ES n = 45	RP n = 45	SEM	<i>P</i> value	Control n = 48	ES n = 48	RP n = 48	SEM	<i>P</i> value
Butyric acid, 4:0	4.03	4.09	4.53	0.64	0.435	6.21	5.50	5.83	0.95	0.457
Caproic acid, 6:0	1.24	1.27	1.25	0.08	0.718	2.12	1.92	1.94	0.15	0.188
Caprylic acid, 8:0	0.93	0.91	0.94	0.06	0.601	1.48	1.71	1.44	0.15	0.075
Capric acid, 10:0	2.28	2.14	2.21	0.09	0.162	2.56 ^a	2.27 ^b	2.49 ^a	0.11	0.010
Lauric acid, 12:0	3.04	2.84	2.82	0.13	0.112	3.04	2.77	3.03	0.14	0.071
Myristic acid, 14:0	9.73	9.34	9.83	0.34	0.150	9.80	9.47	9.52	0.31	0.292
Myristoleic acid, 14:1n-5	1.21	1.23	1.29	0.08	0.331	1.04	1.13	1.11	0.10	0.364
Pentadecanoic acid, 15:0	1.45	1.56	1.52	0.11	0.313	1.24	1.15	1.16	0.11	0.451
Palmitic acid, 16:0	31.05 ^a	28.76 ^b	29.60	0.88	0.010	29.95	28.79 ^a	28.56 ^b	1.04	0.034
Palmitoleic acid, 16:1n-7	2.49	2.26	2.13	0.19	0.063	2.15	2.34	2.24	0.18	0.294
Heptadecanoic acid, 17:0	1.09	1.12	1.15	0.11	0.600	0.85	0.86	0.90	0.09	0.542
Stearic acid, 18:0	9.45	9.95	9.51	0.37	0.184	10.24 ^a	12.06 ^b	11.3 ^a	0.52	0.007
Oleic acid, 18:1n-9	24.62 ^a	26.50 ^b	26.57 ^b	0.90	0.033	24.98	25.86	25.27	0.97	0.151
Linoleic acid, 18:2n-6	4.17	5.11	4.39	0.55	0.090	3.46	4.18 ^a	4.33 ^b	0.41	0.032
CLA ¹	0.63 ^a	0.88 ^b	0.80 ^b	0.07	0.001	0.90	1.03	0.99	0.06	0.060
Arachidic acid, 20:0	1.01	0.82	0.98	0.15	0.229	0.62	0.74	0.86	0.11	0.053
Dihomo- γ -linolenic acid, 20:3n-6	0.87	0.93	1.05	0.19	0.360	1.35	1.35	1.44	0.14	0.499
SFA ²	64.32	61.81	62.76	1.16	0.115	67.05	66.01	66.21	1.08	0.338
UFA	35.68 ^a	38.19 ^b	37.24	1.04	0.025	32.95	33.99	33.79	1.08	0.341
MUFA ³	29.14	30.50	30.41	0.77	0.080	28.00	28.77	28.48	0.92	0.441
PUFA ⁴	6.54	7.69	6.83	0.67	0.083	4.95 ^a	5.22 ^b	5.32 ^a	0.42	0.003
SFA/UFA	1.84	1.66	1.74	0.37	0.323	2.11	1.92	2.00	0.11	0.450
AI	2.12 ^a	1.87 ^b	2.03	0.11	0.048	2.23	2.18	2.15	0.13	0.514
SI	0.81 ^a	0.96 ^b	0.92 ^b	0.05	0.006	0.90	0.81	0.91	0.05	0.065
DFA	45.1	46.3	46.6	1.44	0.298	44.9	44.5	45.8	1.32	0.350

Values are expressed mean value \pm SEM; Means in the same row followed by different in line letters (a and b) are significantly different according to Tukey's test ($P < 0.05$)

CLA – conjugated linoleic acid; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA/UFA – saturated/unsaturated fatty acids ratio; AI – atherogenicity index; SI – spreadability index; DFA – desirable fatty acids; Control – cows were fed total mixed ration; ES – Experimental group 1, total mixed ration with extruded full-fat soya; RP – Experimental group 2, total mixed ration with rapeseed pomace, n-number of milk samples

¹ – cis-9trans-11 18:2 isomer only; ²SFA = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0; ³MUFA = C14:1 + C16:1 + C18:1; ⁴PUFA = C18:2 + C20:3 + CLA

The most well-known PUFAs, CLA_{cis9trans11} was significantly affected by feeding ($P < 0.001$) in all experimental groups during the indoor feeding period, and higher contents of CLA_{cis9trans11} were found in the milk of cows fed a diet supplemented with soya and rapeseed supplements. According to Glasser et al. (2008), total CLAs, CLA_{cis9trans11} were significantly higher by all lipid supplements (rape seed, linseed, sunflower seed and soya bean in the form of seeds, protected seeds or oils). The data of our study showed that pasture-fed cows produced higher concentrations of CLAs in milk fat compared to grass-silage-based fed cows.

Komprda et al. (2000) reported significant increases in total PUFAs when feeding cows a diet of heat-treated rapeseed cakes compared to cows fed a soya bean meal diet. On the other hand, Kudrna and Marounek (2006) and Vesely et al. (2009) reported a more pronounced positive effect of feeding cows extruded soya bean on the above mentioned values than that of feeding them rapeseed cake. These differences probably arise from differences in the composition of the diets (various portions of soybean or rapeseed components). Similarly, the present findings showed that the PUFA content was significantly affected by feeding ($P < 0.01$) during the grazing period and was 2.66% higher in the ES group and 3.6% higher in the RP group compared to that of the cows fed only the farm's total mixed ration.

According to Secchiari et al. (2003), the use of extruded or toasted soya bean supplementation compared to soya bean meal clearly improved the SFA/UFA ratio in milk fat. This is in accordance with our findings that after the inclusion of rapeseed pomace or extruded full-fat soya into the experimental group's diet, the contents of the SFAs/UFAs ratio in milk fat decreased in comparison to that observed in the control diet.

The contents of lauric acid (12:0), myristic acid (14:0), PA and UFAs in milk fat are taken into account in the AI, which 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 the control groups than in the experimental groups, suggesting a more favourable composition of milk from the experimental groups. Similar tendencies were also reported by Vesely et al. (2009) and Krizova et al. (2016). The SI calculated in our experiment was higher in the experimental groups than in the control group and was significantly affected by feeding ($P < 0.01$) during the indoor feeding period. In addition, the DFA content was higher during the indoor feeding period, as well. This is in agreement with the findings of McNamee et al. (2002) who described that rapeseed-based diets resulted in an increased ratio of 18:1n-9/16:0 and therefore produced softer butter fat. Kudrna and Marounek (2006) and Vesely et al. (2009) found only an increase in SI after the inclusion of rapeseed products into the diet. However, it should be taken into account that our experimental group diets, apart from that containing rapeseed pomace, also contained extruded full-fat soya components, which contributed to changes in the FA profile and the above mentioned indexes.

In conclusion, the seasonal alternation between the grass silage-based (indoor feeding period) and the fresh herbage-based (grazing period) diet was identified as an important factor affecting the cow's milk fat composition, moreover, our study confirmed that the composition of the diet is no less important. The nutritional value of milk in the groups fed rations supplemented with extruded full-fat soya or rapeseed pomace was better due to the higher content of UFAs (including MUFAs and PUFAs) and the lower content of PA and SFAs in comparison with the group fed the farm's total mixed ration.

References

- Arnould VMR, Soyeurt H 2009: Genetic variability of milk fatty acids. *J Appl Genet* **50**: 29-39
- Chilliard Y, Ferlay A, Mansbridge RM, Doreau M 2000: Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Ann Zootech* **49**: 181-205
- Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M 2007: Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *Eur J Lipid Sci Technol* **109**: 828-855
- Chouinard PY, Girard V, Brisson GJ 1997: Performance and profiles of milk fatty acids of cows fed full fat, heat-treated soybeans using various processing methods. *J Dairy Sci* **80**: 334-342
- Chouinard PY, Corneau L, Bauman DE, Butler WR, Chilliard Y, Drackley JK 1998: Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. *J Anim Sci* **76**: 233-238
- Claeys WL, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K 2014: Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control* **42**: 188-201
- Commission regulation (EU) No 691/2013 of 19 July 2013 amending Regulation (EC) No. 152/2009 as regards methods of sampling and analysis

- Coppa M, Ferlay A, Chassaing C, Agabriel C, Glasser F, Chilliard Y, Borreani G, Barcarolo R, Baars T, Kusche D, Harstad OM, Verbic J, Golecky J, Martin B 2013: Prediction of bulk milk fatty acid composition based on farming practices collected through on-farm surveys. *J Dairy Sci* **96**: 4197-4211
- Demeyer D, Doreau M 1999: Targets and means for altering meat and milk lipids. *Proc Nutr Soc* **16**: 593-607
- Elgersma A 2015: Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives. *Eur J Sci Technol* **117**: 1345-1369
- Elgersma A, Tamminga S, Ellen G 2006: Modifying milk composition through forage. *Anim Feed Sci Technol* **131**: 207-225
- Faithfull NT 2002: *Methods in Agricultural Chemical Analysis. A Practical Handbook*. CABI Publishing UK
- Feng S, Lock A, Garnsworthy P 2004: Technical note: a rapid lipid separation method for determining fatty acid composition of milk. *J Dairy Sci* **87**: 3785-3788
- Focant M, Mignolet E, Matatu B, Legrand A, Givron C, Vanbelle M 1994: Fatty acid composition of the milk modified by feeding extruded rapeseed to dairy cows. In: *Proc. 45th Annu Mtg of European Association of Animal Production*. Edinburgh Scotland UK 114
- Glasser F, Ferlay A, Chilliard Y 2008: Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. *J Dairy Sci* **91**: 4687-4703
- Gulati SK, Byers E, Ashes JR, Scott TW 1997: Effect of feeding different fat supplements on the fatty acid composition of goat milk. *Anim Feed Sci Technol* **66**: 159-164
- Haug A, Hostmark AT, Harstad OM 2007: Bovine milk in human nutrition: a review. *Lipids Health Dis* **6**: 25
- Komprda T, Dvorak R, Suchy P, Fialova M, Sustova K 2000: Effect of heat treated rapeseed cakes in dairy cow diet on yield, composition and fatty acid pattern of milk. *Czech J Anim Sci* **45**: 325-332
- Krizova L, Hanus O, Spicka J, Samkova E, Frelich J, Richter M, Vesely A, Roubal P 2016: Alternative supplemental mixture for organic dairy herds to maintain desirable milk fatty acid profile throughout the indoor feeding period. *Anim Sci Pap Rep* **34**: 25-40
- Kudrna V, Marounek M 2006: The influence of feeding rapeseed cake and extruded soyabean on the performance of lactating cows and the fatty acid pattern of milk. *J Anim Feed Sci* **15**: 361-370
- Lindmark-Månsson H, Fondén R, Pettersson HE 2003: Composition of Swedish dairy milk. *Int Dairy J* **13**: 409-425
- Markiewicz-Keszyccka M, Czyzak-Runowska G, Lipinska P, Wojtowski J 2013: Fatty acid profile of milk - a review. *Bull Vet Inst Pulawy* **57**: 135-139
- McNamee BF, Fearon AM, Pearce J 2002: Effect of feeding oilseed supplements to dairy cows on ruminal and milk fatty acid composition. *J Sci Food Agr* **82**: 677-684
- Medeiros E, Queiroga R, Oliveira M, Medeiros A, Sabedot M, Bomfim M, Madruga M 2014: Fatty acid profile of cheese from dairy goats fed a diet enriched with castor, sesame and faveleira vegetable oils. *Molecules* **19**: 992-1003
- Micinski J, Zwierzchowski G, Kowalski IM, Szarek J, Pierozynski B, Raistenskis J 2012: The effects of bovine milk fat on human health. *Polish Ann Med* **19**: 170-175
- Nantapo CTW, Muchenje V, Hugo A 2014: Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian x Jersey cross cow milk under a pasture-based dairy system. *Food Chem* **146**: 127-133
- Secchiari P, Antongiovanni M, Mele M, Serra A, Buccioni A, Ferruzzi G, Paoletti F 2003: Effect of fat source on trans C18:1 fatty acid and CLA in milk fat of Italian Friesian cows. *Livest Prod Sci* **83**: 43-52
- Simopoulos AP 2002: The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* **56**: 365-379
- Slots T, Butler G, Leifert C, Kristensen T, Skibsted LH, Nielsen JH 2009: Potentials to differentiate milk composition by different feeding strategies. *J Dairy Sci* **92**: 2057-2066
- Standard GOST 31665 2012: Vegetable oils and animal fats. Preparation of methyl esters of fatty acids. Euro-Asian Council for Standardization, Metrology and Certification (EASC)
- Sukhija PS, Palmquist DL 1988: Rapid method for determination of total fatty-acid content and composition of feed stuffs and feces. *J Agr Food Chem* **36**: 1202-1206
- Timmen H 1990: Characterization of milk fat hardness in farm milk by parameters of fatty acid composition. *Kieler Milchw Forsch* **42**: 129-138
- Ulbricht TL and Southgate DA 1991: Coronary heart disease: seven dietary factors. *Lancet* **338**: 985-992
- Van Soest PJ, Robertson JB, Lewis BA 1991: Methods for dietary fibre, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**: 3583-3597
- Vesely A, Krizova L, Trinacty J, Hadrova S, Navratilova M, Herzig I, Fiser M 2009: Changes in fatty acid profile and iodine content in milk as influenced by the inclusion of extruded rapeseed cake to diet of dairy cows. *Czech J Anim Sci* **54**: 201-209
- Walstra P, Geurts TJ, Noomen A, Jellema A, Boekel MAJS van 1999: *Dairy Technology: Principles of Milk Properties and Processes*. New York: Marcel Dekker