CHANGES IN THE FATTY ACID PROFILE OF COW'S MILK WITH DIFFERENT SOMATIC CELL COUNTS DURING LACTATION

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Abstract. The objective of this study was to determine the effect of lactation stage and the hygienic quality of cow's milk on the fatty acid profile of milk. The experimental materials comprised samples of milk from 56 Polish Holstein-Friesian (PHF) cows. In the 1st, 5th and 10th months of lactation, milk samples were collected for analyses from each cow. Using the MilkoScan FT 120 apparatus, every sample of fresh milk was assayed for proximate chemical composition and the BactoCount apparatus was used for somatic cell count (SCC). The percentage shares of 43 fatty acids (FAs) in the total fatty acid pool were determined by gas chromatography. Ten most common functional FAs were identified. Fatty acids were also divided into the following categories: saturated (SFAs) and unsaturated (UFAs) fatty acids, including monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids, desirable hypocholesterolemic fatty acids (DFAs) and undesirable hypercholesterolemic fatty acids (OFAs). The following ratios were calculated: UFA/SFA, MUFA/SFA, PUFA/SFA and n-6/n-3PUFA.

The obtained results suggest that the stage of lactation affected the concentrations of the analyzed functional FAs in cow's milk. Milk produced by early lactation cows had the most desirable fatty acid profile with respect to FAs delivering health benefits and FA groups, which indicates that milk collected at the first stage of lactation has the best functional properties. SCC had a significant ($P \leq 0.05$) effect on MUFA concentrations, including C 4:0, and on the MUFA/SFA ratio in cow's milk. Increased levels of the majority of functional FAs and FA groups, and higher values of their ratios were noted in milk containing more than 400,000 somatic cells per ml, which could be indicative of mastitis.

Keywords: fatty acids, somatic cell count, mastitis, stage of lactation

Introduction. Apart from essential nutrients, milk contains also chemical compounds that affect the mammary gland and other organs of cow (Malinowski et al., 2008). A particularly important role is played by FAs that are known for their functional properties, including antiatherogenic, anti-inflammatory and antibacterial effects, and provide other health benefits such as lowering blood pressure and stimulating the defence mechanisms of the body. Many FAs aid the prevention and treatment of cancer and Alzheimer's disease, and n-3 FAs are essential dietary nutrients required for optimal growth and development, and they play a vital role in infant nutrition. Milk fat is considered to be the richest natural source of conjugated linoleic acid (CLA) (Baltušnikiene et al., 2008). Milk composition and quality are determined by multiple interrelated factors, including genetic, environmental and physiological factors (Felkner-Poźniakowska et al., 2012). Lactation stage is one of the non-genetic factors that affect the composition, yield and properties of cow's milk. Bovine mastitis remains a recurring and severe problem faced by milk producers (Bernatowicz et al., 2004; Kawai et al., 2003; Petrovski et al., 2006). Mastitis causes vast economic losses that are due to decrease in milk yield, quality and processing suitability as well as to additional costs of veterinary treatment. Mastitis is associated with a reduced milk fat content and decreased dispersion of milk fat globules. Thus, changes in SCC may influence FA concentrations in milk.

In view of the above, the aim of this study was to analyze potential changes in the fatty acid profile of cow's milk as affected by udder health status (SCC) and lactation stage.

Materials and Methods

Materials. The experimental materials comprised samples of milk from 56 Polish Holstein-Friesian (PHF) cows selected from six loose barns with an average herd size of ca. 150 cows, located in the north-eastern Poland. The majority of cows were in their 1st or 2nd lactation, and the average number of lactations of the analyzed cows was 1.81±0.86. Average annual milk per herd was ca. 7,000 kg, and average daily milk yield per cow was 23.56±8.89 kg. Feeding regimes were similar in all barns. Throughout the year, the cows were kept in barns and fed preserved feed mixtures in the Total Mixed Ration (TMR) or Partially Mixed Ration (PMR) system. In the 1st, 5th and 10th month of lactation, during trial milking, milk samples were collected for analyses from each cow. The samples were transported under chilled conditions to a laboratory of the Department of Cattle Breeding and Milk Quality Evaluation at the University of Warmia and Mazury in Olsztyn.

Methods. Samples of fresh milk were assayed for proximate chemical composition (protein, fat, lactose, and dry matter) by infrared spectrophotometry using the MilkoScan FT 120 apparatus (FossElectric), and for SCC by flow cytometry using the BactoCount apparatus (Bentley). Data regarding milk yield, recorded and stored in the SYMLEK system, were provided by the Polish

Federation of Cattle Breeders and Milk Producers (PFHBiPM). Milk fat was extracted by the method proposed by Röse Gottlieb (AOAC, 1990). The percentage shares of 43 FAs were determined by gas chromatography, using the Varian CP 3800 system with a split/splitless injector and a flame-ionization detector (FID). Samples (1 µl) of fatty acid methyl esters were placed on a CP-Sil 88 capillary column (length: 100 m, inner diameter: 0.25 mm). Data were processed using the GALAXIE Chromatography Data System. Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco, Inc. Analyses of samples and reference standards were performed under identical conditions, i.e. carrier gas - helium, injector temperature - 260°C, detector temperature -260°C, initial oven temperature -110°C, raised to 249°C. The following functional FAs were identified: C 4:0 (BA) – butyric acid, C 18:1 *trans* 11 (TVA) – vaccenic acid, C 18:1 *cis* (OA) – oleic acid, C 18:2 (LA) – linoleic acid, CLA – C18:2 *cis 9 trans 11* – conjugated linoleic acid, C 18:3 (LNA) – linolenic acid, C 20:4 (AA) – arachidonic acid, C 20:5 (EPA) – eicosapentaenoic acid, C 22:5 (DPA) – docosapentaenoic acid, C 22:6 (DHA) – docosahexaenoic acid. Fatty acids also were divided into the following categories: saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), desirable hypocholesterolemic fatty acids (DFAs) and undesirable hypercholesterolemic fatty acids (OFAs). The following ratios were calculated: UFA/SFA, MUFA/SFA, PUFA/SFA and n-6/n-3 PUFA.

Statistical analysis. For the purpose of statistical analysis, numerical data were divided into four groups based on SCC in milk (Table 1): group I – up to 100,000 SCC/ml milk (n=56); group II – from 101,000 to 400,000 SCC/ml milk (n=66); group III – from 400,000 to 1 mln SCC/ml milk (n=21); and group IV – over 1 mln SCC/ml milk (n=18). According to the Polish Standard (PN–A–86002:1999), milk from groups I and II was classified to Prime (i.e. containing less than 400,000 SCC/ml). As reported by Hamann (2002), Lindmark-Mansson et al. (2000) and Cheng et al. (2008), milk containing less than 100,000 somatic cells per ml comes from a healthy udder. Thus, milk with the highest hygienic quality was classified as first-class milk. The second experimental factor was lactation stage, and three groups were distinguished in this respect: early lactation (1st month, n=56), mid lactation (5th month, n=49), and late lactation (10th month, n=56). To determine SCC distribution, SCC data were transformed into log10 SCC for each sample before statistical analysis. Numerical data were processed statistically in STATISTICA software (StatSoft, Inc.) by one- and two-way ANOVA with interaction. The significance of differences between mean values in groups was estimated by Fisher's LSD test. ANOVA was performed using the following linear model:

$$Y_{ij} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk},$$

where: Yij - dependent variable, µ - effect of total mean, a_i – effect of somatic cell counts (SCC) (i = I, II, III, IV), b_j – effect of lactation stage (j = early, mid, late lactation), $(ab)_{ij}$ – effect of the SCC x lactation stage interaction, e_{ijk} – random error.

Table 1. **Division into SCC groups based on somatic cells count in milk**

SCC Group	SCC (10^3/ml)	Milk class *according to the Polish Standard PN–A–86002:1999*	Number of samples
I	≤100	Extra class	56
II	101–400		66
III	401–1000	Unclassified milk	21
IV	≥ 1000		18
Total			161

Results and Discussion

An analysis of the proximate composition of milk revealed that milk with the highest hygienic quality had the lowest content of total protein and fat, and the highest lactose content (Table 2). Lactose content decreased with increasing SCC in milk, and differences between groups I and IV were statistically significant (P≤0.05). Higher milk yields were reported in groups I and IV, compared with groups II and III, and the lowest daily milk yield was noted for cows whose milk contained from 400,000 to 1 mln somatic cells per ml. The decrease in lactose concentrations with increasing SCC is consistent with the findings of other authors (Sharif et al., 2007; Philpot and Nickerson, 1991; Bernatowicz et al., 2004). Bernatowicz et al. (2004) and Brzozowski et al. (1999) reported an insignificant decrease in the protein and fat content of milk with SCC of more than 1 mln/ml, which corroborates our results. According to Schallibaum (2001), mastitis causes injury to milk secretory cells in the mammary gland, which interferes with the synthesis of lactose, fat and protein, and affects milk yield. Milk yield and composition also change during lactation. The analysis of the proximate composition demonstrated that the lowest content of total protein and fat, and the highest yield were typical of milk from early stage of lactation (Table 2). Decrease of milk yield and increase of protein and fat content with time after parturition are typical for cows, and were consistent with the findings of other authors (Barłowska et al., 2006; Ostersen et al., 1997; Wielgosz-Groth, 2004).

Table 2. **Basic chemical composition of milk according to the somatic cell count (SCC) and stage of lactation**

Characteristic		Average	SEM	SCC group				Stage of lactation		
				I *n=56*	II *n=66*	III *n=21*	IV *n=18*	early *n=56*	middle *n=49*	late *n=56*
Daily yield (kg)	$\bar{x}$	23.56	1.51	23.86	23.70	21.83	24.16	31.11^{Aa}	23.23^{b}	17.44^{B}
Protein (%)	$\bar{x}$	3.50	0.06	3.36	3.63	3.52	3.46	3.29	3.45	3.84
Fat (%)	$\bar{x}$	4.34	0.13	3.93^{B}	4.76^{A}	4.22	4.29	4.30	4.10	4.68
Lactose (%)	$\bar{x}$	4.70	0.03	4.78^{a}	4.69	4.64	4.61^{b}	4.73	4.66	4.72
Dry matter (%)	$\bar{x}$	13.50	0.15	13.16^{b}	14.04^{a}	13.15^{b}	13.19	13.35	13.15	14.11
Non-fat dry matter (%)	$\bar{x}$	9.25	0.14	9.22	9.42	9.27	8.76	9.30	9.11	9.35
Mean values denoted by different letters in rows within trait are significantly different: a, b – $P < 0.05$; A, B – $P < 0.01$										

Table 3. **Functional fatty acids in milk with different somatic cell count and from different stage of lactation**

Characteristic		average	SEM	SCC group				Stage of lactation			Effect		
				I *n=56*	II *n=66*	III *n=21*	IV *n=18*	early *n=56*	middle *n=49*	late *n=56*	SCC	Stage of lactation	Interaction SCC x Stage of lactation
C 4:0 (BA)	$\bar{x}$	2.51	0.01	2.53	2.52	2.42	2.55	2.69	2.51	2.41	*	**	**
C 18:1 *trans* 11 (TVA)	$\bar{x}$	1.71	0.05	1.66	1.58	1.99	2.14	1.79	1.69	1.69	ns	ns	ns
C 18:1 *cis* (OA)	$\bar{x}$	20.82	0.23	20.05	20.84	21.61	22.03	22.00	19.80	21.41	ns	**	*
C 18:2 (LA)	$\bar{x}$	1.75	0.02	1.78	1.70	1.75	1.90	1.95	1.72	1.67	ns	*	ns
CLA – C18:2 *cis 9 trans 11*	$\bar{x}$	0.63	0.02	0.63	0.58	0.72	0.75	0.57	0.63	0.66	ns	ns	ns
C 18:3 (LNA)	$\bar{x}$	0.43	0.01	0.44	0.41	0.47	0.46	0.44	0.43	0.42	ns	ns	ns
C 20:4 (AA)	$\bar{x}$	0.15	0.002	0.146	0.157	0.153	0.157	0.142	0.155	0.157	ns	ns	ns
C 20:5 (EPA)	$\bar{x}$	0.05	0.001	0.049	0.047	0.051	0.046	0.048	0.048	0.049	ns	ns	ns
C 22:5 (DPA)	$\bar{x}$	0.09	0.001	0.091	0.093	0.093	0.086	0.086	0.092	0.096	ns	ns	ns
C 22:6 (DHA)	$\bar{x}$	0.009	0.001	0.010	0.008	0.009	0.009	0.009	0.008	0.009	ns	ns	ns
Mean values denoted by different letters in rows within trait are significantly different at: a,b - $p \leq 0.05$; A,B - $p \leq 0.01$ Mean values are significantly different at: * $p \leq 0.05$; ** $p \leq 0.01$; ns – non significant													

The predominant functional FA was OA (C 18:1 *cis*) whose average content reached 20.82% (Table 3), followed by BA, TVA and LA, whose concentrations in the analyzed samples were 2.51, 1.71 and 1.75%, respectively. The percentage share of functional FAs in the total fatty acid pool was largely determined by lactation stage. SCC had a significant ($P \leq 0.05$) effect only on the BA (C 4:0) content of milk, but the levels of most functional FAs increased with deteriorating udder health. Only the concentrations of EPA (C 20:5), DPA (C 22:5) and DHA (C 22:6) were lower in milk samples collected in groups III and IV, in comparison with group I. An opposite trend was noted for the remaining functional FAs whose highest levels were observed in milk with the

highest SCC. In a study by Foltys-Kirchnerova et al. (2012), SCC had no significant effect on FA concentrations in cow's milk. However, the maximum SCC in the milk analyzed by the cited authors was 400, 000 per ml, which corresponds to SCC in groups I and II in our study, and we found no significant differences between those groups, either.

The levels of BA (C 4:0, P≤0.01) and OA (C 18:0 *cis*, P≤0.05) were affected by the milk SCC x lactation stage interaction. Lactation stage had a significant effect on the concentrations of the above FAs (P≤0.01) and on LA (C 18:2) content (P≤0.05). The concentrations of BA, OA and LA were lower in milk produced in mid and late lactation. The percentage shares of CLA, AA and DPA increased with progressing lactation. The levels of the other functional FAs decreased with progressing lactation, or remained unchanged, and the noted differences were statistically non-significant.

Stanton et al. (1997), Auldist et al. (1998) and Åkerlind et al. (1999) also demonstrated that the CLA content of milk tended to increase towards the end of lactation. In the cited study, the highest CLA content (0.563%) was noted in late lactation (days 201 to 305), whereas the concentrations of long-chain FAs were highest during the first 100 days of lactation. Paszczyk et al. (2005) analyzed changes in the content of *trans* isomers of C 18:1 and CLA (C 18:2 *cis9 trans11*) in bovine colostrum and milk in early lactation (up to day 82), and reported that milk fat from individual cows contained highly variable amounts of FAs. However, in all cows colostrum fat had a lower average FA content than milk fat. In the cited study, CLA content was highest (0.77%) on lactation day 17, whereas the average CLA content during the first 82 days of lactation reached 0.42%, and it was lower than the value noted in our study in early lactation (0.57%). According to Stoop et al. (2009), changes in FA concentrations in milk during lactation are associated with changes in the energy status of cows.

A decrease in the concentrations of SFAs and OFAs was noted in milk samples with a high SCC (Table 4). The percentage shares of the other FA groups increased with deteriorating udder health. SCC had a significant (P≤0.05) effect on MUFAs and the MUFA/SFA ratio. Our results are partially consistent with the findings of other authors. Bernatowicz et al. (2004) demonstrated that enhanced transport of bioactive components from blood to milk with increasing SCC was characteristic of cows with mastitis. Everson (1980) reported that milk with SCC of 700,000 and greater was rancid. Rancid and lipolyzed flavours are related to the breakdown of fats to various short-chain FAs. Unlike in our study, Kuczyńska (2011) noted lower SFA levels and higher UFA concentrations in milk with lower SCC, which is highly desirable in view of the functional properties of milk.

Table 4. **Groups of fatty acids in milk with different somatic cell count and from different stage of lactation**

Characteristic		average	SEM	SCC group				Stage of lactation			Effect		
				I *n=56*	II *n=66*	III *n=21*	IV *n=18*	early *n=56*	middle *n=49*	late *n=56*	SCC	Stage of lactation	Interaction SCC x Stage of lactation
SFA	$\bar{x}$	67.68	0.27	68.65	67.83	66.24	65.99	66.74	68.79	66.84	ns	ns	ns
UFA	$\bar{x}$	32.33	0.27	31.34	32.19	33.80	34.07	33.32	31.22	33.16	ns	ns	ns
MUFA	$\bar{x}$	28.48	0.25	27.48	28.45	29.73	29.91	29.40	27.36	29.34	*	*	ns
PUFA	$\bar{x}$	3.86	0.04	3.86	3.74	4.07	4.15	3.93	3.86	3.82	ns	ns	ns
UFA/SFA	$\bar{x}$	0.49	0.006	0.463	0.484	0.520	0.522	0.512	0.459	0.505	ns	ns	ns
MUFA/SFA	$\bar{x}$	0.43	0.006	0.406	0.428	0.458	0.459	0.453	0.402	0.447	*	*	ns
PUFA/SFA	$\bar{x}$	0.06	0.00	0.057	0.056	0.062	0.063	0.060	0.057	0.058	ns	ns	ns
n-6	$\bar{x}$	1.90	0.02	1.92	1.86	1.90	2.06	2.09	1.87	1.82	ns	*	ns
n-3	$\bar{x}$	0.58	0.01	0.59	0.55	0.62	0.60	0.58	0.58	0.57	ns	ns	ns
n-6/n-3 PUFA	$\bar{x}$	3.54	0.06	3.52	3.55	3.47	3.71	3.81	3.50	3.43	ns	ns	ns
OFA		57.79	0.34	58.93	58.12	55.96	55.02	56.34	59.20	56.84	ns	*	ns
DFA		42.23	0.35	41.07	41.90	44.07	45.05	43.72	40.80	43.17	ns	*	ns
Mean values denoted by different letters in rows within trait are significantly different at: a,b - p≤0.05; A,B - p≤0.01 Mean values are significantly different at: * p≤0.05; ** p≤0.01; ns – non significant													

There was no interaction between SCC and lactation stage for any of the analyzed FA groups. Lactation stage had a significant (P≤0.05) effect on the concentrations of MUFAs, n-6 FAs, DFAs and OFAs, and the MUFA/SFA ratio. The concentrations of PUFAs, n-6 FAs and the n-6/n-3 PUFA ratio decreased with progressing lactation, and the lowest values were noted in milk produced in late lactation. Milk produced in mid lactation was characterized by the lowest levels of UFAs, including MUFAs (27.36%), and DFAs (40.80%), and by the highest concentrations of SFAs and OFAs, which exert adverse health effects. Our results corroborate the findings of Miciński et al. (2012) who reported the lowest SFA concentrations in milk samples collected from early

lactation cows. In the above study, SFA levels in milk were highest (64.71 g/100 g) at peak lactation (day 90), and then decreased with decreasing milk production. Unlike in our experiment, the cited authors observed an increase in the PUFA content of milk with progressing lactation. An opposite trend was reported by Nałęcz-Tarwacka (2006) and Barłowska et al. (2008), who noted the highest PUFA concentrations in milk produced by early lactation cows, which is consistent with our findings.

In the present study, lactation stage had no significant effect on n-3 FAs and the n-6/n-3 PUFA ratio. This finding supports previous research conducted by Nałęcz-Tarwacka [2006] and Kuczyńska (2001).

Summary and Conclusions

Increased levels of the majority of functional FAs and FA groups, and higher values of their ratios were noted in milk containing more than 400,000 somatic cells per ml, which could be indicative of mastitis.

Lactation stage had a significant effect on the concentrations of functional FAs in milk, which decreased with progressing lactation. Milk produced by early lactation cows had the most desirable fatty acid profile with respect to FAs delivering health benefits and FA groups.

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