THE EFFECT OF CATTLE BREED AND LACTATION STAGE ON NUTRIENT CONCENTRATIONS IN MILK AND THE FATTY ACID PROFILE OF MILK FAT

Wiesław Sobotka[1], Jan Miciński[2], Paulius Matusevičius[3], Birutė Staniškienė[3], Magdalena Sobiech[1], Grzegorz Zwierzchowski[2], Elwira Fiedorowicz[1], Renata Pietrzak-Fiećko[4]

[1]*Department of Animal Nutrition and Feed Science, Faculty of Animal Bioengineering*
University of Warmia and Mazury in Olsztyn, Poland
[2]*Department of Cattle Breeding and Milk Quality Evaluation, Faculty of Animal Bioengineering*
University of Warmia and Mazury in Olsztyn, Poland
[3]*Veterinary Academy of Lithuanian University of Health Sciences, Kaunas, Lithuania*
[4]*Department of Commodities and Food Analysis, Faculty of Food Sciences*
University of Warmia and Mazury in Olsztyn, Poland
e-mail: wsob@uwm.edu.pl; micinski@uwm.edu.pl

Abstract. The aim of this study was to determine the effect of cattle breed (Holstein-Friesian – HF, Jersey – JE, HF x JE) and lactation stage (days 160, 220 and 280) on nutrient concentrations in milk and the fatty acid profile of milk fat. An analysis of lactation stage on the chemical composition of milk revealed that the concentrations of dry matter, protein and fat tended to increase until day 220. The content of dry matter, ash and fat in milk increased further until day 280. Cattle breed had a significant effect on the levels of saturated fatty acids (SFA) in milk fat. The concentrations of long-chain fatty acids (LCFA) in bovine milk were considerably higher (66.26% on average), compared with short-chain fatty acids (SCFA) (9.11% on average). SCFA content tended to decrease with the progress of lactation. The differences among the analyzed stages of lactation were significant at $p \leq 0.05$ for $C_{4:0}$ and $C_{10:0}$ fatty acids. In the group of LCFA, the content of $C_{12:0}$ and $C_{18:0}$ fatty acids was higher on day 160 of lactation, and the content of $C_{14:0\ iso}$ and $C_{16:0}$ fatty acids was higher towards the end of lactation, on day 280. The average concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in milk fat were 21.97% and only 2.79%, respectively. The highest MUFA ($C_{10:1}$, $C_{12:1}$, $C_{14:1}$, $C_{18:1\ cis9}$) content of milk was noted in HF cows, and the lowest in HF x JE crossbreeds. HF x JE cows were characterized by the highest content of only trans-isomers of monounsaturated fatty acid $C_{18:1\ t6+t9+t10+t11}$. The concentrations of n-3 fatty acids were highest in HF cows. HF cows had also a desirable n-6/n-3 fatty acid ratio, at 3.92. The difference between this value and those noted in the other breeds was significant at $p \leq 0.01$.

Keywords: cows milk, nutrients, fatty acids, cattle breed, lactation stage.

Introduction. Milk, which is a rich source of high-quality nutrients, plays an important role in human nutrition. The chemical composition of bovine milk and the protein to fat ratio are constantly being modified in order to meet consumer preferences. The optimal protein to fat ratio in cow's milk should be 1:1. Such a value could be achieved by decreasing fat content or increasing protein content, which is not an easy task since milk fat and protein concentrations are determined by genetic factors and environmental conditions as well as cow's age and lactation stage (Kamieniecki *et. al*., 1998, Litwińczuk *et al*., 2000). One of the primary goals of the dairy industry has always been to improve the technological properties of milk, including its chemical composition. Milk processing suitability is significantly affected by the proportions of milk components. The quality of milk intended for consumption and processing varies subject to cattle breed (Barłowska *et al*., 2009).

Milk fat is the main source of dietary energy and the most easily digested fat of animal origin in the human diet. According to Barłowska *et al.* (2006), in Poland the average fat content of cow's milk oscillates around 3.7%, but it may vary widely (2.8% – 8.1%) depending on cattle breed, nutritional regime and lactation stage.

Milk fat contains various fatty acids, including SCFA which are readily absorbed from the digestive tract and metabolized in the liver, and which do not contribute to fat deposition (Pfeuffer, 2001). The proportion of essential PUFA in milk fat is also an important consideration. Recent research shows that PUFA, including conjugated linoleic acid (CLA), deliver health benefits (Reklewska *et al.*, 2003, Cichosz, 2007). However, it seems that the fatty acid profile of milk fat differs between cattle breeds, and it may also be affected by lactation stage.

The aim. In view of the above, the objective of this study was to determine the effect of cattle breed (Holstein-Friesian – HF, Jersey – JE, HF x JE) and lactation stage on nutrient concentrations in milk and the fatty acid profile of milk fat.

Materials and Methods. The experiment was conducted at the Research and Experimental Station in Bałdy, owned by the University of Warmia and Mazury in Olsztyn. The experimental materials comprised 63 dairy cows divided into three groups (Table 1).

The animals were fed a partially mixed ration, PMR Mix-1 until day 160 of lactation and PMR Mix-2 from day 160 until the end of lactation. PMR Mix-1 contained maize silage, haylage, Soypass protein feed and Pro Agro KT-19 supplementary feed. PMR Mix-2 was based on maize silage and haylage, supplemented with Soypreme protein feed. The cows were fed twice daily, at 7.00 a.m.

and 2.00 p.m., with a feed cart. In addition, all groups were fed concentrates in feeding stations, depending on milk yield (with the use of the animal identification system). The animals were kept in a free-stall barn with deep litter.

Table 1. **Experimental design**

Specification	Experimental groups								
Cattle breed	Holstein-Friesian (HF)			Jersey (JE)			HF * JE		
Lactation stage, day	160	220	280	160	220	280	160	220	280
Number of cows whose milk was sampled	33	33	33	14	14	14	16	16	16

Milk samples were collected over the winter feeding season (October 2010 - March 2011), during test milking, from cows of different breeds, and at different lactation stages (Table 1). The samples were cooled to 4^0C and were transported to the analytical laboratory of the Department of Animal Nutrition and Feed Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, to determine the chemical composition of milk, including the content of: dry matter (Polish Standard, 2002), ash (Polish Standard, 1976), protein – by the Kjeldahl method, using the Kjeltec 2200 Auto Distillation unit (Polish Standard, 2000).

Milk fat was extracted by the Röse-Gottlieb method (AOAC, 2000) to determine its fatty acid profile. Fatty acid methyl esters were prepared by the method proposed by Peisker (Żegarska *et. al*., 1991). Fatty acids were methylated with a mixture of methanol-chloroform-concentrated sulfuric acid. Methyl esters were separated by gas chromatography using the Varian CP-3800 chromatograph equipped with a flame ionization detector (FID) and a CP-Sil 88 capillary column with a length of 50 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium the flow rate was 1.2 ml/min. The temperature of the column, detector and injector was 50^0C (increase of 5^0C/min to 200^0C), 250^0C and 225^0C, respectively. Fatty acids were identified by comparing their retention times with those of methyl ester reference standards (Sigma-Aldrich). Chromatography data were recorded using the Star Chromatography Workstation software.

The results were processed by two-way ANOVA for orthogonal designs. Least squares means (LSM) and standard deviations (SD) were calculated. The significance of differences between means was estimated by Duncan's test. All calculations were performed using STATISTICA ver. 9.0 PL software (StatSoft, 2009).

Results and Discussion. An analysis of the proximate chemical composition of milk revealed significant ($p \leq 0.01$) differences between breeds in the content of dry matter, protein and fat (Table 2). The protein content of milk ranged from 3.66% in HF cows to 3.98% in JE cows. Milk from JE cows had the highest content of fat (5.52%) and dry matter (15.08%). Differences between JE cows and the other breeds were significant at $p \leq 0.01$. Milk from HF and HF x JE cows was characterized by lower concentrations of fat and dry matter, compared with JE cows, by 1.83% and 1.22%, and by 2.32% and 1.40%, respectively. The ash content of milk was comparable in all breeds, and it ranged from 0.52% in JE cows to 0.59% in HF cows.

Table 2. **Essential nutrient content of milk subject to cattle breed and lactation stage**

Specification	Statistical measures	Experimental factor						Analysis of variance, p≤			
		Breed (R)			Lactation stage, day (F)			SEM	Breed (R)	Lactation stage (F)	Interaction (R x F)
		HF	JE	HF x JE	160	220	280				
Dry matter, %	LSM	12.76^A	15.08^B	13.68^C	13.52	13.77	14.18	0.18	<0.01	0.07	0.46^{xx}
	SD	0.85	0.59	0.67	1.37	1.22	0.94				
Ash, %	LSM	0.59	0.52	0.54	0.55^a	0.52^a	0.60^b	0.01	0.06	0.02	0.17
	SD	0.09	0.09	0.07	0.08	0.11	0.04				
Protein, %	LSM	3.66^A	3.98^B	3.66^A	3.70^a	3.91^b	3.72^a	0.04	<0.01	0.04	0.12
	SD	0.24	0.36	0.18	0.22	0.41	0.20				
Fat, %	LSM	3.69^A	5.52^B	4.30^C	4.34	4.45	4.67	0.15	<0.01	0.47	0.20^x
	SD	0.60	0.55	0.76	1.10	1.12	0.75				
Protein / Fat	LSM	0.99^a	0.72^b	0.85^b	0.85^a	0.88^b	0.80^c	0.03	<0.01	<0.01	0.04
	SD	0.19	0.29	0.14	0.24	0.21	0.14				

SEM – standard error of the mean; Means followed by different letters differ significantly:
a,b,c – $p \leq 0.05$; A, B, C – $p \leq 0.01$; x – $p \leq 0.05$; xx – $p \leq 0.01$

Many authors have demonstrated that the content of major milk components is higher in JE cows than in HF cows (Litwińczuk *et al.,* 1998, Barłowska *et al.,* 2006, Miciński *et al.,* 2006, Antkowiak *et al*., 2007, Miciński *et al.,* 2008). Our results, similarly as the findings of Kuczaj *et al.* (2001) and Litwińczuk *et al.* (1998), show that cattle breed has a significant ($p \leq 0.01$) effect on the chemical composition of milk.

Cattle breed had a significant influence on the protein to fat ratio, which was most desirable (0.99) in milk from HF cows where protein content was similar to fat content, and the latter was below the average reference value for the HF breed. In JE and HF x JE cows, the protein to fat ratio was lower, at 0.72 and 0.85, respectively.

An analysis of lactation stage on the chemical composition of milk revealed that the concentrations of dry matter, protein and fat tended to increase until day 220. The content of dry matter, ash and fat in milk increased further until day 280. Our results corroborate the findings of other authors (Miciński *et al.*, 2006, Antkowiak *et al.* 2007).

In the present study, neither cattle breed nor lactation stage had a significant positive effect on CLA concentrations in milk. CLA had a 0.43% – 0.47% share of the total MUFA pool. Similar values were reported by Gardzina *et al.* (2005) and Felkner-Poźniakowska *et al.* (2012).

The protein to fat ratio in milk from cows of the analyzed breeds was most desirable on lactation day 220, at 0.88. On days 160 and 280, it reached 0.85 and 0.80, respectively.

The data in Table 3 show that cattle breed had a significant effect on SFA levels in milk fat. The concentrations of LCFA in bovine milk were considerably higher (66.26% on average), compared with SCFA (9.11% on average). The highest average SFA concentrations were found in milk from JE cows (77.08% of the total SCFA and LCFA pool), followed by HF x JE cows (76.14%) and HF cows (72.90%).

Table 3. **Concentrations of saturated fatty acids in milk subject to cattle breed and lactation stage** (% total fatty acid pool

Saturated fatty Acids (*SFA*)	Fatty acid	Statistical measures	Experimental factor						Analysis of variance, p≤			
			Breed (R)			Lactation stage, day (F)			SEM	Breed (R)	Lactation stage (F)	Interaction (R x F)
			HF[1]	JE[1]	HF x JE[1]	160	220	280				
Short-chain Fatty acids (*SCFA*)	$C_{4:0}$	LSM	2.30^{A}	2.46^{B}	2.67^{C}	2.53^{a}	2.54^{a}	2.35^{b}	0.05	0.01	0.16	0.57^{xx}
		SD	0.29	0.35	0.28	0.48	0.23	0.18				
	$C_{6:0}$	LSM	1.98	1.98	2	2.02	1.96	1.99	0.02	0.83	0.50	0.90^{xx}
		SD	0.20	0.13	0.06	0.22	0.06	0.08				
	$C_{8:0}$	LSM	1.37	1.36	1.29	1.4	1.3	1.31	0.02	0.18	0.08	0.03
		SD	0.16	0.10	0.09	0.16	0.11	0.07				
	$C_{10:0}$	LSM	3.39^{Aa}	3.44^{Ab}	3.10^{Bc}	3.45^{a}	3.16^{b}	3.31^{a}	0.05	0.02	0.07	0.11
		SD	0.45	0.22	0.33	0.39	0.45	0.18				
	Total	**LSM**	**9.20**	**9.08**	**9.06**	**9.40**	**8.96**	**8.85**	-	-	-	-
Long-chain fatty acids (*LCFA*)	$C_{12:0}$	LSM	4.00^{a}	4.14^{Ab}	3.68^{Bb}	4.10^{a}	3.74^{b}	3.97^{c}	0.07	0.00	0.04	0.02
		SD	0.55	0.24	0.44	0.43	0.61	0.24				
	$C_{13:0}$	LSM	0.14^{aA}	0.13^{b}	0.12^{B}	0.13	0.13	0.13	0.00	0.00	0.46	0.03
		SD	0.02	0.01	0.01	0.01	0.01	0.02				
	$C_{14:0}$ *iso*	LSM	0.15^{a}	0.19^{b}	0.19^{b}	0.16^{a}	0.19^{b}	0.18^{b}	0.01	0.02	0.12	0.43^{xx}
		SD	0.05	0.03	0.03	0.05	0.05	0.01				
	$C_{14:0}$	LSM	12.26^{a}	12.27^{a}	11.77^{b}	12.1	11.72	12.48	0.11	0.06	0.01	0.13
		SD	0.84	0.44	0.78	0.61	0.86	0.52				
	$C_{15:0}$	LSM	1.28	1.28	1.25	1.22	1.28	1.3	0.02	0.70	0.17	0.53^{xx}
		SD	0.14	0.1	0.12	0.14	0.12	0.8				
	$C_{16:0}$ *iso*	LSM	0.32	0.34	0.35	0.31	0.36	0.34	0.01	0.27	0.15	0.31^{x}
		SD	0.08	0.04	0.05	0.06	0.08	0.02				
	$C_{16:0}$	LSM	33.76^{A}	37.80^{B}	38.03^{C}	35.7	35.89	37.89	0.6	0.00	0.11	000
		SD	3.15	4.36	3.14	2.87	3.05	5.58				
	$C_{17:0}$	LSM	0.59	0.59	0.58	0.57	0.6	0.59	0.01	0.85	0.34	0.20^{x}
		SD	0.06	0.03	0.06	0.05	0.05	0.04				
	$C_{18:0}$	LSM	11.02	11.08	10.93	11.54	11.19	10.26	0.26	0.96	0.06	0.01
		SD	1.6	1.6	2.2	2.21	1.21	1.57				
	$C_{20:0}$	LSM	0.18	0.18	0.18	0.18^{a}	0.19^{a}	0.16^{b}	0.01	0.95	0.02	0.00
		SD	0.03	0.04	0.03	0.04	0.03	0.04				
	Total	**LSM**	**63.7^{A}**	**68.00^{B}**	**67.08^{B}**	**66.01^{a}**	**65.29^{a}**	**67.30^{a}**	-	-	-	-
Total		**LSM**	**72.90^{A}**	**77.08^{B}**	**76.14^{B}**	**75.41^{a}**	**74.25^{b}**	**76.26^{a}**	-	-	-	-

SEM – standard error of the mean; Means followed by different letters differ statistically significantly:
a, b, c – $p \leq 0.05$; A, B, C – $p \leq 0.01$; x – $p \leq 0.05$; xx – $p \leq 0.01$

Butyric acid $C_{4:0}$, a member of the SCFA family, is a potent antineoplastic and antibacterial agent. In the present study, the highest butyric acid content (2.67%) was determined in milk from HF x JE crossbreeds, and the lowest (2.30%) in milk from HF cows. The above difference was significant at $p \leq 0.01$. A similar trend was observed with regard to LCFA, i.e. $C_{14:0}$ and $C_{16:0}$, whose content was higher in JE and HF x JE cows than in HF cows.

SCFA content tended to decrease with the progress of lactation. Differences between the analyzed stages of lactation were significant at $p \leq 0.05$ for $C_{4:0}$ and $C_{10:0}$ fatty acids. In the group of LCFA, the content of $C_{12:0}$ and $C_{18:0}$ fatty acids was higher on day 160 of lactation, and the content of $C_{14:0}$ and $C_{16:0}$ fatty acids was higher towards the end of lactation, on day 280.

Unsaturated fatty acids (UFA) are most valuable of all fatty acids contained in milk fat, due to their health-promoting properties, including antineoplastic: $C_{18:1\ cis9}$, $C_{18:2(cis9\ trans11)}$ (CLA), $C_{20:4}$, $C_{20:5}$, $C_{22:6}$, antiantherogenic: $C_{18:1cis9}$, $C_{18:2\ cis9\ trans11}$ (CLA), $C_{20:4}$, $C_{20:5}$, antihypertensive: $C_{20:5}$, $C_{22:6}$, anti-inflammatory: $C_{20:4}$, $C_{20:5}$, $C_{22:6}$, and boosting immunity: $C_{18:2\ cis9\ trans11}$ (CLA) (Parodi, 2004, Williams C.M., 2000).

Table 4. **Concentrations of unsaturated fatty acids in milk subject to cattle breed and lactation stage** (% total fatty acid pool)

Unsaturated fatty acids (UFA)	Fatty acid	Statis-tical measu-res	Experimental factors						Analysis of variance, p≤			
			Breed (R)			Lactation stage, day (F)			SEM	Breed (R)	Lactation stage (F)	Interaction (R x F)
			HF	JE	HF x JE	160	220	280				
Monounsaturated fatty acids (MUFA)	$C_{10:1}$	LSM	0.32^{A}	0.30^{A}	0.28^{B}	0.32	0.30	0.29	0.01	0.01	0.08	0.00
		SD	0.05	0.03	0.02	0.05	0.03	0.02				
	$C_{12:1}$	LSM	0.12^{A}	0.12^{A}	0.10^{B}	0.12	0.11	0.11	0.00	0.00	0.18	0.00
		SD	0.02	0.01	0.01	0.02	0.02	0.01				
	$C_{14:1}$	LSM	1.01^{A}	1.00^{A}	0.84^{B}	0.94	0.92	1.00	0.03	0.00	0.20	0.00
		SD	0.21	0.12	0.12	0.23	0.16	0.10				
	$C_{16:1}$	LSM	1.27	1.26	1.29	1.19^{a}	1.34^{b}	1.30^{b}	0.04	0.97	0.30	0.17
		SD	0.4	0.23	0.19	0.24	0.35	0.25				
	$C_{17:1}$	LSM	0.37	0.36	0.37	0.34^{a}	0.37^{b}	0.39^{c}	0.01	0.45	0.00	0.51^{xx}
		SD	0.05	0.02	0.06	0.04	0.06	0.02				
	$C_{18:1\ t6+t9+t10+t11}$	LSM	0.98^{a}	1.09^{a}	1.16^{b}	1.03	1.15	1.05	0.04	0.17	0.42	0.58^{xx}
		SD	0.35	0.19	0.15	0.27	0.33	0.12				
	$C_{18:1\ c9}$	LSM	19.46^{A}	16.49^{Ba}	17.73^{Bb}	17.88	18.49	17.42	0.37	0.00	0.38	0.10
		SD	2.49	2.12	1.89	2.69^{A}	1.67^{B}	2.91^{A}				
	Total	**LSM**	$\mathbf{23.53^{a}}$	$\mathbf{20.62^{b}}$	$\mathbf{21.77^{c}}$	**21.82**	**22.68**	**21.56**	-	-	-	-
Polyunsaturated fatty acids (PUFA)	$C_{18:2}$	LSM	2.05^{A}	1.35^{B}	1.59^{C}	1.92^{A}	1.70^{B}	1.39^{C}	0.08	0.00	0.00	0.54^{xx}
		SD	0.5	0.45	0.5	0.61	0.45	0.49				
	$C_{18:2\ c9t11}$ (CLA)	LSM	0.44^{a}	0.43^{a}	0.47^{b}	0.44	0.47	0.45	0.22	0.09	0.12	0.11
		SD	0.1	0.09	0.09	0.11	0.09	0.09				
	$C_{18:3}$	LSM	0.57^{A}	0.45^{B}	0.47^{B}	0.50	0.50	0.49	0.01	0.00	0.82	0.02
		SD	0.1	0.1	0.08	0.08	0.09	0.13				
	$C_{20:4}$	LSM	0.16^{a}	0.13^{b}	0.15^{a}	0.15	0.15	0.15	0.00	0.01	0.84	0.27^{x}
		SD	0.03	0.03	0.03	0.03	0.02	0.04				
	$C_{20:5}$	LSM	0.04	0.04	0.04	0.04	0.04	0.04	0.00	0.49	0.08	0.05
		SD	0.01	0.01	0.01	0.01	0.01	0.01				
	Total	**LSM**	$\mathbf{3.26^{A}}$	$\mathbf{2.40^{B}}$	$\mathbf{2.72^{C}}$	$\mathbf{3.05^{A}}$	$\mathbf{2.86^{B}}$	$\mathbf{2.52^{C}}$	-	-	-	-
Total		**LSM**	$\mathbf{26{,}79^{Aa}}$	$\mathbf{23.02^{Bb}}$	$\mathbf{24.49^{Bc}}$	**24.87**	**25.54**	**24.08**	-	-	-	-

SEM – standard error of the means; Means followed by different letters differ statistically significantly:
a, b, c – $p \leq 0.05$; A, B, C – $p \leq 0.01$; x – $p \leq 0.05$; xx – $p \leq 0.01$

The data in Table 4 indicate that cattle breed had a significant effect on UFA levels in milk fat. The average MUFA and PUFA concentrations in milk fat samples collected from cows of all three breeds were 21.97% and only 2.79%, respectively. The highest MUFA ($C_{10:1}$, $C_{12:1}$, $C_{14:1}$, $C_{18:1\ cis\ 9}$) content of milk was noted in HF cows, and the lowest in HF x JE crossbreeds. The noted differences were significant at $p \leq 0.01$. HF x JE cows were characterized by the highest content of only isomers of $C_{18:1\ t6+t9+t10+t11}$. ($p \leq 0.05$). Higher concentrations of PUFA ($C_{18:2}$, $C_{18:3}$, $C_{20:4}$) were also found in HF cows. In HF x JE cows, the only PUFA present in large quantities was CLA ($p \leq 0.05$).

Recent years have witnessed an increasing interest in the trans and cis isomers of UFA and conjugated fatty acids. Efforts have been made to explain their molecular and genetic mechanisms of action as well as their role in promoting human health. Numerous physiological properties and health benefits have been attributed to CLA (Sugano *et al*., 1999, Kuczyńska *et al*., 2009).

The only acids of the UFA family whose concentrations increased over lactation were $C_{16:1}$ and $C_{17:1}$ ($p \leq 0.01$). Lactation stage had no significant influence on the MUFA content of milk. Among PUFA, differences between lactation stages were observed only with in respect of $C_{18:2}$ ($p \leq 0.01$).

The effect of lactation stage on the chemical composition of cow's milk, noted in the present experiment, was similar to that reported by other authors (Miciński *et al*., 2006, Antkowiak *et al.* 2007).

Neither cattle breed not lactation stage had a significant, positive effect on CLA concentrations in milk. CLA had a 0.43% - 0.47% share of the total MUFA pool, which is consistent with the findings of Gardzina *et al.* (2005) and Felkner-Poźniakowska *et al.* (2012).

As shown in Table 5, the concentrations of n-3 fatty acids were highest in HF cows. HF cows had also a desirable n-6/n-3 fatty acid ratio, at 3.92. The difference between this value and those noted in the other breeds was significant at $p \leq 0.01$.

SFA concentrations increased with the progress of lactation, MUFA levels remained stable, and PUFA content decreased. The n-6/n-3 fatty acid ratio was most favourable on lactation day 160, and it decreased at successive lactation stages to reach 3.02 on day 280 (Table 5; $p \leq 0.01$). The most desirable n-6/n-3 fatty acid ratio, at 3.92, was observed in HF cows. Similar results were reported by Matwijczuk *et al.* (2009) who studied the following cattle breeds: Black-and-White HF, Red-and-White HF and Simmental. Barłowska *et al.* (2006) demonstrated that SFA concentrations increase, while UFA levels decrease in milk with a high fat content.

Table 5**. Concentrations of saturated fatty acids (SFA), unsaturated fatty acids (MUFA and PUFA), and n-3 and n-6 fatty acids in milk subject to cattle breed and lactation stage** (% total fatty acid pool)

Fatty acids	Statistical measures	Experimental factors						Analysis of variance, p≤			
		Breed (R)			Lactation stage, day (F)			SEM	Breed (R)	Lactation stage (F)	Interaction (R x F)
		HF	JE	HF x JE	160	220	280				
SFA	LSM	72.90	77.08	76.14	75.41	74.25	76.26	0.94	0.47	0.15	0.29^{x}
	SD	3.16	3.53	2.5	3.59	2.28	3.4				
MUFA	LSM	23.53	20.62	21.77	21.82	22.68	21.56	0.93	0.40	0.26	0.12
	SD	2.67	3.04	2.3	2.96	1.9	2.9				
PUFA	LSM	3.26^{A}	2.40^{B}	2.72^{C}	3.05	2.86	2.52	0.87	0.10	0.02	0.54^{xx}
	SD	0.66	0.56	0.55	0.73	0.63	0.57				
n-3	LSM	0.60^{A}	0.47^{B}	0.50^{B}	0.52	0.53	0.52	0.09	0.00	0.01	0.39^{x}
	SD	0.1	0.08	0.08	0.07	0.1	0.14				
n-6	LSM	0.37	0.36	0.37	0.34^{Aa}	0.37^{Ab}	0.39^{Bc}	0.01	0.45	0.00	0.51^{xx}
	SD	0.05	0.02	0.06	0.04	0.06	0.02				
n-6/n-3	LSM	3.92^{A}	3.17^{B}	3.68^{C}	4.07^{A}	3.67^{B}	3.02^{C}	0.01	0.03	0.00	0.52^{xx}
	SD	0.91	0.55	0.95	1.04	0.72	0.35				

SEM – standard error of the means; Means followed by different letters differ statistically significantly:
a, b, c – $p \leq 0.05$; A, B, C – $p \leq 0.01$; x – $p \leq 0.05$; xx – $p \leq 0.01$

Conclusions

1. Milk from JE cows had the highest content of fat (5.52%) and dry matter (15.08%). Differences between JE cows and the other breeds were significant at $p \leq 0.01$. Cattle breed had a significant ($p \leq 0.01$) effect on the protein to fat ratio, which was most desirable (0.99) in milk from HF cows, characterized by a low fat content, similar to protein content. In JE and HF x JE cows, the protein to fat ratio was lower, at 0.72 and 0.85, respectively. An analysis of lactation stage on the chemical composition of milk revealed that the concentrations of dry matter, protein and fat tended to increase until day 220. The content of dry matter, ash and fat in milk increased further until day 280.

2. Cattle breed had a significant effect on SFA levels in milk fat. The concentrations of LCFA in bovine milk were considerably higher (66.26% on average), compared with SCFA (9.11% on average). SCFA content tended to decrease with the progress of lactation. Differences between the analyzed stages of lactation were significant at $p \leq 0.05$ for $C_{4:0}$ and $C_{10:0}$ fatty acids. In the group of LCFA, the content of $C_{12:0}$ and $C_{18:0}$ fatty acids was higher on day 160 of lactation, and the content of $C_{14:0\ iso}$ and $C_{16:0}$ fatty acids was higher towards the end of lactation, on day 280.

3. The average MUFA and PUFA concentrations in milk fat were 21.97% and only 2.79%, respectively. The highest MUFA ($C_{10:1}$, $C_{12:1}$, $C_{14:1}$, $C_{18:1\ cis\ 9}$) content of milk was noted in HF cows, and the lowest in HF x JE crossbreeds. HF x JE cows were characterized by the highest content of only trans isomers of monounsaturated fatty acid ($C_{18:1\ t6+t9+t10+t11}$).

4. The concentrations of n-3 fatty acids were highest in HF cows. HF cows had also a desirable n-6/n-3 fatty

acid ratio, at 3.92. The difference between this value and those noted in the other breeds was significant at $p \leq 0.01$.

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