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IMPACT OF LACTATION STAGE AND MILK PRODUCTION ON MILK FAT FATTY ACIDS RATIO

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ABSTRACT

Milk fat is, from a nutritional point of view, of the negative value because of the prevalent content of saturated fatty acids with high atherogenic index. Intake of milk fat in the diet is important because of the content of monounsaturated fatty acids, acting favorably against cardiovascular diseases and especially of essential fatty acids: linoleic, alpha-linolenic and conjugated linoleic acid (CLA), which is found only in meat and milk of ruminants.

The analysis of relations of fatty acids in milk fat to qualitative-production parameters of milk shows that the correlations of fatty acids with lactation stage and qualitative-production parameters of milk are quite weak in dairy cows with stable type of nutrition in form of whole-the-year feeding mixed feed ration in lowland agricultural area. Coefficients r>0.3 for the values of lactation sum were observed at monounsaturated fatty acids (MUFASC), r = 0.467 for days, 0.307 for milk, 0.353 for fat, and 0.340 for protein total production. The most important fatty acids, as far as their content is concerned, C12:0, C14:0, C16:0, C18:0, C18:1n9, the ratio of which is higher than 5 % in milk fat and they represent together about 75 % of milk fat, show no significant relations either to sum or to daily production parameters or to the content of basic components in milk. With the exception of C16:0, palmitic acid (30.93 ± 4.81 % in milk fat), which has negative relation to daily milk (r = -0.404) and protein (r = -0.345) production. This acid has positive relation to the content of fat in milk (r = 0.444) and negative relation to the content of lactose in milk (r = -0.311). CLA showed negative correlation with daily fat production (r = -0.407) and content of fat in milk (r = -0.269) and F/P index (r = -0.420).

Key words: milk; fat; fatty acid; stage of lactation; correlation

INTRODUCTION

Milk fat is from nutritional point of view evaluated negatively because of prevalent content of saturated fatty acids with atherogenic effect, as well as content of undesirable trans isomers, but this is negligible in milk fat compared with other fats. The occurrence of fatty acid trans isomers, which are put into connection with the incidence of cardio-vascular diseases, is an important factor that influences the effect of fat on health, because they affect negatively the ratio of HDL and LDL cholesterol similarly to hypercholesterolemic acids (Mensink, 2005). However, this is a problem mainly in hydrogenated fat. Trans-isomers of mono-unsaturated fatty acids are created in rumen during biohydrogenation process. This is a desirable process, which enables rise of chains of essential fatty acids, linoleic and alpha-linolenic, and the main of them, the acid delta 11 trans C18:1 is the precursor of conjugated linoleic acid (CLA), which occurs only in meat and milk of ruminants. These chains are precursors of biologically active substances – hormones and enzymes. Milk products are the main source of CLA, which is considered to be the functional component of foods with positive influence on health. The occurrence of other trans-isomers of monounsaturated fatty acids (MUFA) in milk fat is minimal with marginal detectability in relation to other fatty acids. The intake of milk fat in nutrition is important for the content of fatty acids increasing its biological value. Important are mainly unsaturated fatty acids in cis-configurations, first of all oleic acid C18:1n6 cis, which acts positively against cardio-vascular disorders (Haug, 2007). Positive

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property of milk fat is also its good ratio, 1: (1.16-4), of omega-3 (n3) and omega-6 (n6) fatty acids (Colomb *et al*., 2004). Assessment of milk fat importance in nutrition should be done by complex studies, not only on the basis of effect of individual types of acids, when they are studied separately.

The influence of lactation stage on milk fat composition corresponds with metabolic origin of fatty acids, when acids synthesized *de novo* are lower at the beginning of lactation than in later stages as a result of negative energy balance, and with their growth in the course of lactation decrease fatty acids with longer chain. There were found changes during the first third of lactation, which cease gradually up to 10 weeks or to first 100 days. During the beginning of lactation the content of lower acids synthesized *de novo* increases, and the content of higher acids from deposit fat decreases (Garnsworthy *et al.*, 2006). Komprda *et al.* (2001) studied representation of fatty acids in first third of lactation. Content of myristic acid rose significantly and content of stearic acid decreased irrespective of feeding ration. Results from studies of composition changes in fatty acids (Kirchnerová *et al*., 1988) showed that during the first 18 weeks of lactation the total content of fatty acids $C_{18:0}$ and $C_{18:1}$ decreased from 40 to 30 %, and content of fatty acids $C_{14:0}$ and $C_{16:0}$ increased from 35 to 45 % of the total content of fatty acids, whereas the content of linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids was relatively stable. Changes in milk fat composition during early lactation were connected with different intensity of nutrition during the preparation for lactation during the period of drying off.

The objective of this work was to study relations among lactation stage and qualitative and production parameters of milk and representation of fatty acids of milk fat in dairy cows with stable type of nutrition by the system of round-the-year feeding the mixed feed ration in lowland agricultural area.

MATERIAL AND METHODS

The herds with stable type of nutrition by round-the-year feeding the mixed feed ration based on maize silage with no farm differences were selected for this study. The dairy cows were at first lactation on different number of days of lactation evenly distributed in the interval of 22 - 309 days. The data on their milk performance were processed on the basis of milk recording. The group of dairy cows from herds with round-the-year feeding ration has quite high average daily production of milk, fat and proteins ([27.78 ± 6.27, 1.03 ± 0.29 and 0.85 ± 0,17] $kg.day^{-1}$) with low variability (20 %). The milk was sampled from the whole amount of milked milk at regular milk recording. Single milk samples collected from individual dairy cows (n = 100) on 6 farms (n = 15–30) in lowland agricultural area were analysed for physiological and biochemical parameters as mentioned below. They were also analyzed for fatty acids in milk fat using gas chromatography.

Analysis of fatty acids by gas chromatography

Milk fat was isolated from lyophilized milk samples by extraction in petroleum ether according to Röse-Gottlieb, then it was re-esterified by methanol potassium hydroxide solution, and methyl esters of fatty acids were extracted by hexane. Methyl esters of fatty acids were analysed by gas chromatography (apparatus GC Varian 3800, Techtron, USA), using FID detector in capillary column Omegawax 530; 30m. Irregular temperature gradient from 40 to 240°C, injection and detection at 250°C were used. Nitrogen flow rate was 6 $ml.min^{-1}$. In the chromatography record 54 fatty acids inclusive of particular isomers were identified by standard reference sample of milk fat and analytical standards Supelco, followed by GCMS analysis. Their representation was expressed relatively in percents (%). Groups of fatty acids and their abbreviation as well as calculated indexes were created according to traditional structural-chemical and nutrition criteria in line with studies cited in References.

Analyses of milk samples

Content of fat, proteins and lactose was determined by infrared analyser Milkoscan FT 120 (FOSS Electric), with DID detector (diode array = diode field in whole red spectrum) according to ISO 9622: 1999 Whole milk – Determination of milk fat, protein and lactose content – Guidance on the operation of mid-infrared instruments. Somatic cells count (SCC) was determined in apparatus Somacount 150 (Bentley Instruments), on the principle of through-flow cytometry, according to STN EN ISO 13366-1: 2008. Temperature of milk freezing (TMF) was determined in thermistor cryoscopic apparatus Cryostar (Funke Gerber), according to the norm ISO 5764: 2002 Milk – Determination of freezing point. Content of urea was determined photocolorimetrically with Ehrlich's agent at 530 nm wave length.

Mathematic-statistical evaluation of results

Results of analyses were processed by variation-statistical methods using the Statgraphics software. Following statistical characteristics were calculated: arithmetical mean (x), minimum and maximum value, standard deviation (sx), variation coefficient (v %). The test of two means agreement (t-test - type for uneven variances) was used to determine significance of difference. Coefficients of linear correlation (r) were calculated to express relations among the studied parameters, and their statistical significance was tested.

The results did not show waves or change in course in relation to the number of lactation days, which would indicate dissection of lactation into linear parts. On this basis we studied the relations among the detected parameters by means of linear regression for the total number of lactation days.

RESULTS AND DISCUSSION

If we are seeking for possibilities how to improve the profile of fatty acids in milk fat, it is necessary to evaluate interrelations among production parameters in set of dairy cows as well as the qualitative properties of milk, which reflect their condition and spectrum of fatty acids. Changes in qualitative or production parameters and composition of milk fat during lactation were not very great with round-the-year feeding the mixed feed ration in lowland agricultural area. This stability was manifested also in relations among the studied parameters.

Tables 1 and 2 show that correlation coefficients are quite low. Correlations among volatile fatty acids (VFA) and values of lactation sum reached the limit of statistical significance ($P<0.01$), when $r < -0.3$. These coefficients are decreasing with the length of chain in individual acids C4:0, C6:0, C8:0 and C10:0. They are the highest in absolute value with butyric acid (C4:0), namely $r = -0.418$ to the number of lactation days, -0.342 to the amount of produced milk, -0.322 to the amount of fat, and -0.372 to the amount of proteins. Fatty acids with short chains up to C10 come from the biosynthesis in the milk gland. Acetic acid is the building stone, which arises at fermentation processes in rumen. In the process of reduction condensation it creates a prolonged chain in form of Acetyl-CoA, creating higher and higher fatty acids with even number of carbon atoms in this way

Table 1: Correlation coefficients for parameters of production and quality of milk to groups of fatty acids in milk fat

	Days of lactation	Milk kg	Fat kg	Protein kg	Milk kg.D^{-1}	Fat kg.D^{-1}	Protein kg.D^{-1}	F/P index	Fat g.100g^{-1}	Protein of milk	Lactose	SCC .10^3.ml^{-1}	TFM -m°C	Urea mg.l^{-1}
	D	Sum of lactation			Daily production				Milk composition			Milk quality		
SAFASC	-0,184	-0,203	-0,073	-0,204	0,046	0,198	0,023	0,122	0,041	-0,167	-0,121	0,167	0,092	-0,009
SAFAMC	0,013	-0,210	0,085	-0,165	**-0,416**	0,050	**-0,357**	**0,376**	**0,446**	0,170	**-0,306**	0,046	-0,269	-0,148
SAFALC	0,003	0,161	-0,017	0,141	0,268	-0,020	0,237	-0,096	-0,128	-0,052	0,230	-0,124	0,027	-0,048
SAFA	-0,162	-0,296	-0,009	-0,269	-0,183	0,217	-0,170	**0,370**	0,337	-0,044	-0,269	0,148	-0,111	-0,139
VFA	**-0,332**	-0,298	-0,224	**-0,316**	0,170	0,266	0,096	0,188	0,014	**-0,323**	-0,057	0,173	0,098	0,018
HCHFA	0,052	-0,158	0,149	-0,112	**-0,382**	0,067	**-0,308**	**0,316**	**0,408**	0,192	**-0,316**	0,081	-0,189	-0,135
BCFA	0,206	0,038	0,127	0,073	**-0,392**	-0,233	**-0,333**	0,038	0,137	0,185	0,112	0,083	0,191	**0,245**
MUFASC	**0,467**	**0,307**	**0,353**	**0,340**	-0,265	-0,243	-0,168	**-0,364**	-0,151	**0,330**	-0,119	-0,012	-0,057	-0,160
MUFAMC	0,105	0,049	-0,024	0,048	-0,170	-0,145	-0,162	-0,077	-0,050	0,063	0,141	-0,023	0,038	0,121
MUFALC	0,087	0,256	-0,029	0,224	0,254	-0,151	0,227	**-0,334**	**-0,336**	-0,016	0,277	-0,136	0,148	0,169
MUFA	0,128	0,282	-0,006	0,252	0,224	-0,179	0,205	**-0,367**	**-0,352**	0,011	0,281	-0,140	0,148	0,167
PUFA	**0,352**	0,284	0,118	0,291	-0,205	**-0,418**	-0,165	-0,243	-0,087	0,278	0,068	-0,154	-0,211	-0,127
USFA	0,162	0,296	0,009	0,269	0,183	-0,217	0,170	**-0,370**	**-0,337**	0,044	0,269	-0,148	0,111	0,139
SCFA	-0,147	-0,176	-0,047	-0,175	0,027	0,176	0,011	0,094	0,030	-0,140	-0,125	0,161	0,085	-0,020
MCFA	0,022	-0,205	0,082	-0,160	**-0,427**	0,037	**-0,368**	**0,367**	**0,439**	0,174	-0,292	0,043	**-0,264**	-0,137
LCFA	0,099	0,259	-0,014	0,231	0,240	-0,156	0,216	-0,295	-0,290	0,000	0,273	-0,149	0,096	0,098
desC14	**0,550**	**0,474**	**0,330**	**0,488**	-0,133	**-0,345**	-0,059	**-0,548**	**-0,340**	**0,337**	0,080	-0,139	-0,084	-0,146
desC16	0,177	0,283	0,052	0,258	0,132	-0,128	0,125	-0,292	-0,270	0,037	0,257	-0,069	0,177	0,197
desC18	0,111	0,113	-0,013	0,104	-0,060	-0,197	-0,045	-0,286	-0,225	0,086	0,076	-0,028	0,191	**0,317**
n6	**0,316**	**0,354**	0,078	**0,328**	0,017	**-0,377**	0,006	**-0,464**	**-0,403**	0,065	0,247	-0,168	-0,039	-0,149
n3	0,146	-0,039	0,057	0,008	**-0,392**	-0,201	**-0,317**	0,231	0,391	**0,342**	-0,198	-0,048	**-0,336**	-0,092
n6/n3	0,015	0,185	-0,034	0,130	0,353	-0,026	0,272	**-0,446**	**-0,557**	-0,281	0,273	0,003	**0,249**	-0,018
AI	-0,023	-0,148	0,113	-0,122	-0,173	0,174	-0,138	0,251	0,268	0,039	**-0,306**	0,141	-0,120	-0,162
EMK	**0,365**	**0,306**	0,122	**0,309**	-0,190	**-0,433**	-0,156	-0,277	-0,127	0,262	0,090	-0,154	-0,208	-0,138

(Melcher 1975, Jenkins and Mcguire 2006, Bauman *et al.*, 2006).

Coefficients r > 0.3 for the values of lactation sum were observed at monounsaturated fatty acids (MUFASC), r = 0.467 for days, 0.307 for milk, 0.353 for fat, and 0.340 for proteins, caused mainly by myristic acid (C14:1), which is out of all evaluated acids in the closest relation to the level of production for the past lactation period; r = 0.597 for days, 0.481 for milk, 0.431 for fat and 0.508 for proteins. In connection with it is also opposite to other desaturation indices, the extraordinary relation of des-C14 (r = 0.545 days, 0.474 milk, 0.330 fat, 0.488 proteins) to sum parameters. C14:1 has also a high correlation coefficient to the content of proteins in milk r = 0.409.

The most important fatty acids, as far as content is concerned, C12:0, C14:0, C16:0, C18:0, C18:1n9, the ratio of which is higher than 5 % in milk fat and they represent together about 75 % of milk fat, show no significant relations either to sum or to daily production parameters or to the content of components in milk. With the exception of C16:0, palmitic acid (30.93 ± 4.81 % in milk fat), which has negative relation to daily milk production (r = -0.404) and proteins (r = -0.345). This acid has positive relation to the content of fat in milk (r = 0.444) and negative relation to the content of lactose

Table 2: Correlation coefficients for parameters of production and quality of milk to ratio of fatty acids in milk fat

	Days of lactation	Milk kg	Fat kg	Protein kg	Milk kg.D^{-1}	Fat kg.D^{-1}	Protein kg.D^{-1}	F/P index	Fat g.100g^{-1}	Protein of milk	Lactose	SCC .10^{3}.ml^{-1}	TFM -m°C	Urea mg.l^{-1}
	D	Sum of lactation			Daily production				Milk composition			Milk quality		
C4:0	**-0,418**	**-0,342**	**-0,322**	**-0,372**	0,260	0,287	0,152	0,180	-0,043	-0,401	-0,028	0,107	0,093	0,046
C6:0	**-0,353**	**-0,318**	-0,259	-0,341	0,178	0,258	0,088	0,185	-0,010	-0,363	-0,055	0,161	0,072	-0,005
C8:0	-0,288	-0,280	-0,184	-0,293	0,113	0,242	0,054	0,198	0,047	-0,282	-0,068	0,189	0,100	0,007
C10:0	-0,197	-0,199	-0,084	-0,202	0,074	0,218	0,051	0,162	0,068	-0,182	-0,072	0,209	0,104	0,009
C12:0	-0,020	-0,084	0,069	-0,068	-0,066	0,120	-0,034	0,089	0,103	0,006	-0,120	0,173	0,083	-0,036
C14:0	0,196	0,109	0,278	0,141	-0,179	-0,009	-0,090	-0,024	0,116	0,228	-0,175	0,056	0,085	-0,001
C15:0	0,144	0,026	0,124	0,043	-0,208	-0,134	-0,176	-0,323	-0,212	0,120	-0,242	-0,053	-0,192	-0,219
C16:0	0,013	-0,205	0,090	-0,161	**-0,404**	0,057	**-0,345**	**0,374**	**0,444**	0,170	**-0,311**	0,045	**-0,274**	-0,158
C17:0	-0,176	**-0,310**	-0,242	-0,291	-0,298	-0,114	**-0,314**	0,097	0,069	-0,039	-0,113	-0,031	-0,190	-0,030
C18:0	-0,008	0,153	-0,026	0,132	0,277	-0,012	0,242	-0,097	-0,138	-0,069	0,239	-0,121	0,035	-0,044
C20:0	**0,301**	**0,330**	0,253	**0,343**	0,004	-0,164	0,062	-0,107	0,070	0,323	-0,033	-0,181	-0,131	-0,104
C14:0i	-0,165	**-0,315**	-0,035	-0,270	**-0,324**	0,117	-0,283	**0,477**	**0,464**	0,037	-0,039	0,124	0,087	**0,227**
C15:0ai	0,254	0,097	0,200	0,120	**-0,309**	-0,190	-0,257	-0,162	-0,050	0,147	-0,018	0,110	0,126	0,025
C16:0i	0,068	-0,146	0,075	-0,095	**-0,448**	-0,049	**-0,379**	**0,474**	**0,563**	0,243	-0,063	0,127	0,119	**0,357**
C17:0i	0,053	0,140	-0,068	0,119	0,101	-0,170	0,074	**-0,303**	**-0,350**	-0,103	**0,348**	-0,090	0,212	0,128
C14:1	**0,597**	**0,480**	**0,431**	**0,508**	-0,213	**-0,325**	-0,105	**-0,529**	-0,275	**0,409**	-0,021	-0,113	-0,039	-0,151
C15:1	0,094	-0,148	0,088	-0,091	**-0,475**	-0,064	**-0,384**	**0,366**	**0,511**	**0,328**	-0,155	0,097	-0,052	0,088
C16:1n7cis POA	0,158	0,100	0,079	0,107	-0,170	-0,097	-0,139	-0,044	0,026	0,147	0,074	-0,038	0,009	0,108
C16:1	0,157	0,095	-0,064	0,085	-0,152	**-0,301**	-0,163	-0,280	-0,269	-0,006	0,241	-0,035	0,047	-0,006
C17:1n7cis	-0,277	-0,259	**-0,322**	-0,274	-0,008	0,053	-0,070	0,165	0,027	-0,221	0,101	0,073	0,091	0,214
C18:1n9cis OA	0,077	0,249	-0,030	0,218	0,259	-0,134	0,235	**-0,313**	**-0,314**	-0,010	0,271	-0,131	0,159	0,184
C18:1	0,143	0,272	-0,056	0,229	0,193	-0,298	0,137	**-0,528**	**-0,553**	-0,111	**0,319**	-0,169	0,029	-0,008
C20:1n9cis	**0,355**	**0,411**	**0,319**	**0,421**	0,009	-0,159	0,087	-0,211	-0,065	0,261	0,151	-0,151	0,049	0,132
C18:2n6cis LA	**0,321**	**0,373**	0,091	**0,345**	0,045	-0,358	0,034	**-0,474**	**-0,417**	0,062	0,259	-0,165	-0,042	-0,154
C18:3n3cisALA	0,167	-0,013	0,057	0,029	**-0,386**	-0,236	**-0,319**	0,196	**0,351**	**0,327**	-0,185	-0,044	**-0,357**	-0,110
C18:2 9,11 CLA	**0,394**	**0,427**	0,179	**0,415**	-0,034	**-0,407**	-0,007	**-0,419**	-0,269	0,248	0,136	-0,138	-0,047	0,021
C20:4n6cis ETA	0,026	-0,069	-0,071	-0,068	-0,226	-0,203	-0,233	-0,085	-0,062	0,002	0,069	-0,091	0,119	-0,042
C20:4n3cis	0,104	-0,030	0,139	0,028	-0,225	0,067	-0,115	0,270	**0,443**	**0,387**	-0,233	-0,057	-0,061	0,061
C20:5n3cisEPA	0,029	-0,110	0,064	-0,060	-0,283	0,021	-0,202	**0,322**	**0,462**	**0,318**	-0,251	0,028	-0,228	-0,022

in milk (-0.311), which manifested also in the relation of saturated fatty acids with medium chain length (SAFAMC) to the content of fat (r = 0.446) and lactose (r = -0.306) in milk. The relation to lactose was transferred also into the relation to TMF (r = -0.274), to which lactose has generally a positive relation. This fatty acid with medium chain usually does not change markedly its content in milk fat in the course of lactation, which was manifested also in lower milk yield, where more marked changes in other acids took place, also showed that it has a similar function among fatty acids as lactose among milk components; its production capacity, which is determining for milk amount, becomes exhausted at higher production. Hanuš *et al.* (2010) was engaged in the study of relations of fatty acids, which are important for health, to milk components. In their study they did not observe significant relations between the most important saturated palmitic fatty acid C16:0 and milk components. Higher saturated fatty acids from C_{18} come predominantly from blood plasma, into which they got from feed or from reducing depot fat of dairy cow. Fatty acids from C_{12} to C_{16} can be of both origins. Odd fatty acids arise by prolongation of propionyl-CoA instead of acetyl-CoA. Branched chain fatty acids arise by prolongation of chain that arose by oxidative deamination of branched amino acids (valine – isoaminovaleric acid, leucine – isoaminocaproic acid, isoleucine – anteisoaminocaproic acid). This construction can be realised by microorganisms in forestomachs at the synthesis of microbial fat, or by cells of secretion epithelium in milk gland (Melcher 1975, Jenkins and Mcguire 2006, Bauman *et al.*, 2006).

CLA and isomers C16:1 and C18:1 have negative correlation with daily fat production (for CLA -0.407) and content of fat in milk (for CLA -0.269) and index F/P (for CLA -0.420); for C18:1 -0.553 to fat in g/100g, and -0.528 to the F/P index. They indicate in this way that they are of another origin than other higher unsaturated fatty acids, which are resorbed from blood of dairy cow, namely that they are probably synthesized *de novo* in secretion epithelium, and their proportion in milk decreases with the increase of fat content in milk. CLA content in milk fat increases during lactation, most closely in connection with increasing total milk production, r = 0.427 and proteins r = 0.415. CLA biosynthesis increases in spite of gradual exhaustion of body fat reserves. Hanuš *et al.* (2010) observed statistically significant relations of CLA proportion in milk fat to fat content (r = 0.379; P<0.01) and to the content of lactose (r = -0.542; P<0.001). Total production of fat and lactose plays probably an essential role in these relations. Milk fat unsaturated fatty acids $C_{18:1}$ and $C_{16:1}$ can originate from resorption of the feed fat but more unsaturated fatty acids originate from own synthesis by milk gland, because unsaturated fatty acids from feed lipids are hydrogenated in forestomachs of ruminants. Higher organisms are able to incorporate double bonds in molecule by means of dehydrogenating enzyme system and reactions of chain growth (Melcher 1975, Jenkins and Mcguire 2006, Bauman *et al.*, 2006).

The content of n6 acids (mostly C18:2n6LA) increases slightly in connection with sum of lactation and decreases with the rise of daily production of fat, and it decreases significantly with increasing content of fat in milk (r = -0.403) and more markedly with F/P index (r = -0.464; P<0.01). It manifested itself also in marked decrease in the ratio n6/n3, mainly with fat content (r = -0.557; P<0.001). The less marked increase in n3 acids supported it also. Marked decrease in the content of polyunsaturated fatty acids (PUFA) (r = -0.418) and essential fatty acids (EFA) (r = -0.433) with the increase of daily fat production is the result. Hanuš *et al.* (2010) observed statistically significant relations of total sum of polyunsaturated fatty acids to the content of fat in milk (r=0.321; P<0.05) and to the content of lactose in milk (r=0.458; P<0.01). However, within this group it is important to evaluate separately n6 and n3 fatty acids.

The relation of alpha linolenic acid (ALA) with the content of dry matter (r = 0.411) is positive, but it is negative with the absolute TMF value (expressed in –m°C) (r = -0.357). This is contradictory, because if dry matter is rising, TMF should expectedly rise also. This shows complexity of TMF parameter as well as the fact that this theory has no unambiguous confirmation by results; TMF is more influenced by other milk properties, e.g. acidity, which influences dissociation of salts in milk.

SCC, which has low variation coefficient in the system with round-the-year feeding ration, and a standard maximum value to $400.10^3.ml^{-1}$ that was kept during the study, has in this span no significant influence on representation of fatty acids in milk fat. Similarly, Hanuš *et al.* (2010) did not observe significant relations between SCC and content of fatty acids in milk fat.

Content of urea has a slightly positive relation to the content of branched chain fatty acids (BCFA) (0.245), mainly to C14:0i (0.227) and C16:0i (r=0.357), which can be explained by the origin of these isoacids from deaminated chains of amino acids from proteins, which are used as source of energy, the urea being created at the same time. Both these acids have negative correlation (r = -0.324 and -0.448) with rising daily production of milk, as well as with rising production of milk proteins (r = -0.283; -0.380), which means that their content is higher with the higher content of milk fat with which they have positive correlation (r = 0.464; 0.562). Also the odd C15:1 decreases with daily production of milk (r = -0.475) and proteins (r=-0.384), which is related to equal origin of its chain.

CONCLUSION

The analysis of relations of fatty acids in milk fat to qualitative-production parameters of milk shows that the correlations of fatty acids with lactation stage and qualitative-production parameters of milk are quite weak in dairy cows with stable type of nutrition in form of whole-the-year feeding mixed feed ration in lowland agricultural area. Changes in milk fat composition are caused by the change in the ratio of *de novo* and depot fatty acids. Relation of fatty acids to the evaluated parameters depends on their metabolic origin and neither acid nor group underlies the specific influence of the studied parameters, by the means of which it would be possible to influence its proportion in milk fat. Therefore, it is not possible to influence some group or desirable fatty acid e.g. CLA, without the influence on total milk fat.

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