Journal of Microbiology, Biotechnology and Food Sciences

Kirchnerová et al. 2013 : 2 (Special issue 1) 1164-1174

REGULAR ARTICLE

IMPACT OF LACTATION STAGE ON MILK FAT FATTY ACIDS PROFILE IN GRAZING DAIRY COWS

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ABSTRACT

The aim of the paper was to extend the knowledge about correlation of current fatty acids (FAs) profile of cow milk fat at herds of cows (n=134) at summer pasture period in mountain dairy farms in Slovakia to milk production and quality parameters. The FAs composition of individual milk was determined by GC-MS, where 54 FAs were identified. Saturated fatty acids (SAFA) (70.48 ± 4.04% in the milk fat) show in the first third of lactation highly significant positive correlation coefficients (r> 0.45, P <0.01) with all indicators of milk production (days, the total amount of milk fat and protein in kg). Monounsaturated fatty acids (MUFA) (26.26 ± 3.59%) have to the total milk production significant indirect relationship. Their content decreases with the rise of the total amount (kg) of produced fat (r=-0.426), protein (r=-0.494), milk (r=-0.514), and with the increasing number of days of lactation (r=-0.583, P <0.001). Polyunsaturated fatty acids (PUFA) (3.26 ± 0.069%) show negative correlation coefficients to total amount of produced milk, fat, protein (kg) and the number of days in lactation from r=-0.468 to r=-0.485 (P <0.01). Grazing of dairy cows at mountain farms has a better value of the composition of milk fat from a health perspective, but at the account of lower production.

Keywords: milk, fat, fatty acids, lactation

INTRODUCTION

Fatty acid composition of milk fat in relation to grazing dairy farming method studied **Kraft *et al.*, (2003)** and **Leiber *et al.*, (2005)**. Milk fat of grazing cows had higher amounts of polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA). **White *et al.*, (2001)** found in dairy pasture higher levels of fatty acids with carbon numbers in C14 and CLA. Seasonal increase in PUFA (2.86> 2.56%) and twice as high CLA content (1.00> 0.49%) during the summer grazing in the winter season compared to feeding hay or silage confirmed **Sloniewski *et al.*, (2005)**. The authors concluded that milk produced in the grazing season is biologically more valuable than milk produced in winter. Similarly **Frelich *et al.*, (2009)** dealt with the composition of milk fat on low input mountain farms. The content of long-chain (> C16), mono- and polyunsaturated FAs in the milk fat was higher in the grazing period (49.22, 31.69 respctively 4.69 g/100 g total FAs) than in the indoor period (42.25, 27.55 respectively 4.15 g/100 g total FAs, $P < 0.01$). Ecological organic farming in the UK **(Ellis *et al.*, 2005)** had compared to conventional breeding significantly lower MUFA (26.19 and 27.63%) and higher PUFA (3.89 and 3.33%) and higher omega-3 linolenic acid content. CLA (0.65 and 0.58%) and total SAFA (68.13 a 67.25%) content was insignificantly higher. For both types of fatty acids showed a strong seasonal effect and during summer grazing their levels increase. CLA content was increased in both types of farms from 0.4 to 0.8% from April to June. **Soják *et al.*, (2009)** in the study of fatty acids in milk fat of sheep found that the by transition from dry feed to pasture is achieved the increasing content of isomer 9-cis ,11-trans (CLA), trans-vaccenic acid (TVA), α-linolenic acid (ALA).

The objective of this work was to study relations among lactation stage and quantitative production parameters of milk and representation of fatty acids of milk fat in dairy cows on pasture.

MATERIAL AND METHODS

The milk samples were taken in total from 134 cows at summer pasture period. At 6 dairy farms in the mountain area in field below High Tatra in the altitude about 800 m about the sea level (breed Pinzgau, Holstein, Simental). The dairy cows were at first lactation on different number of days of lactation evenly distributed in the interval of 10 – 111 days. The data on their milk performance were processed on the basis of milk recording. The milk was sampled from the whole amount of milked milk at regular milk recording. Single milk samples were analyzed for fatty acids in milk fat using gas chromatography. Results of analyses were processed by variation-statistical methods of the system programme Statgraphics.

Analysis of fatty acids by gas chromatography

Milk fat was isolated from lyophilised 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 (**Samková *et al.,* 2009**). 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. In the chromatography record were identified 54 fatty acids inclusive of particular isomers. Their representation was expressed relatively in percent's (%). 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 works given in References.

RESULTS AND DISCUSSION

In the grazing dairy cows from farms in mountain areas (tab. 1) in comparison with dairy cows with year-round food ration in lowland areas the individual milk had a higher content of mono-and polyunsaturated fatty acids (26.26 and 3.26%), and the higher level of essential fatty acids EFA, 2.96%, from which the most important is CLA, 0.54%. Also, omega-3 acids had higher representation, 0.65%, and in this group was lower atherogenic index AI = 3.01. Grazing dairy cows have lower daily production indicators (average daily production of milk, fat, and protein was significantly ($P < 0.0001$) higher in farms with year-round food ration (27.78, 1.03, respectively 0.85 kg.D-1) versus the farms with grazing (21.81, 0.90, respectively 0.71 kg.D-1) **(Kirchnerová *et al.,* 2012).**

We evaluated the correlation relations of production parameters of dairy cows and a range of fatty acids in a mountain pasture farm system. Due to course changes by fatty acids in milk fat, which is more pronounced in the first third of lactation, we assessed by linear regression analysis of indicators of quality and milk production for the first 111 days of lactation (tab. 2 and 3).

Table 1 Basic parameters of milk production and fatty acids composition.

		Pasture (n=134)	**Whole year feed mix** (n=100)
Milk ($kg.D^{-1}$)	**x**	**21.81**[b]	**27.78**[a]
	Sx	6.34	6.27
Fat kg (D^{-1})	**x**	**0.90**[b]	**1.03**[a]
	Sx	0.33	0.29
Protein ($kg.D^{-1}$)	**x**	**0.71**[b]	**0.85**[a]
	Sx	0.20	0.17
SAFA	**x**	**70.48**[b]	**71.24**[b]
	Sx	4.04	6.28
VFA	**x**	**9.47**[b]	**11.62**[a]
	Sx	2.16	4.14
HCHFA	**x**	**48.40**	**47.24**
	Sx	4.27	5.89
BCFA	**x**	**2.02**[a]	**1.75**[b]
	Sx	0.33	0.21
MUFA	**x**	**26.26**[a]	**25.84**[b]
	Sx	3.59	5.82
PUFA	**x**	**3.26**[a]	**2.92**[b]
	Sx	0.69	0.77
n6	**x**	**2.06**	**1.95**
	Sx	0.32	0.43
n3	**x**	**0.65**	**0.58**
	Sx	0.24	0.41
n6/n3	**x**	**3.46**[b]	**4.40**[a]
	Sx	1.02	1.80
AI	**x**	**3.01**[b]	**3.08**[b]
	Sx	0.63	0.86
EFA	**x**	**2.96**	**2.85**
	Sx	0.61	0.75
CLA	**x**	**0.54**[a]	**0.39**[b]
	Sx	0.23	0.19

[a, b] Statistically significant difference (P<0.001)
Legend: SAFA-Saturated fatty acids (FA), VFA- Volatile FA, HCHFA-Hypercholesterolaemic FA, BCFA Branched chain FA, MUFA-Monounsaturated FA, PUFA-Polyunsaturated FA, AI-Atherogenic index, EFA-Essential FA, CLA conjugated linoleic acid

Saturated fatty acids SAFA (70.48 ± 4.04%), which are considered undesirable part of milk fat, reported in the first third of lactation highly significant positive correlation coefficients (r> 0.45, P <0.01) with all indicators of milk production so far in the lactation. The higher the number of days of lactation at the day of sampling, the higher the content of saturated fatty acids in the milk fat (r = 0.599). An important role is played by the quantity of produced milk (0.537), fat (0.454) and protein (0.517). This relationship of SAFA consists of their part, consisting of fatty short chain acids SAFASC (28.82 ± 4.23%). Among the correlation coefficients for parameters of lactation (where are all r> 0.368, P <0.01) have the closest relationship to the number of days of lactation r = 0.657 , P <0.001. The sum of the fatty acids of medium chain length SAFAMC (33.49 ± 2.94), in to which we calculate C16: 0

- C17: 0, including izo and anteizo forms, is less significant relationship to the total amount of already produced milk.

With lactation production highly positively correlated acids C12:0 and C14:0, and moderately positively correlated C16:0 are summarized in the amount of hypercholesterolemic saturated fatty acids HCHFA (48.40 ± 4.27%). This file, which actually makes up half of the fatty acids of milk fat has all coefficients relating to the total production in the range from r = 0.450 for fat in kg to r = 0.584, P <0.001 for lactation day. Atherogenic index (3.01 ± 0.63) calculated from the content of these acids, from which the C14:0 is considered quadrupled effective, with count of lactation days is in highly statistically significant correlation (0.631, P <0.001), as well as the amount of produced milk (0.570), fat (0.470) and protein (0.550). Next part of the SAFA group is the group of long-chain acids SAFALC (8.17 ± 1.69%) with a saturated chain from C18 above. It was in a significant negative relationship with the number of lactation days (-0.607, P <0.001), and to produced quantity of milk, fat and protein, when all r <-0.33, P <0.01. Especially in the first third of lactation body fat reserves from which comes part of fatty acids built in milk fat are degraduated, and gradually extends biosynthesis of de novo fatty acids with shorter chains. In the first third of lactation grows the share of cholesterolemic fatty acids and increases the Atherogenic index.

Self-assessed branched chain fatty acids BCFA (2.02 ± 0.33%) are related to previous milk production negatively, the narrowest to fat (-0.512, P <0.001). Within this group we also observed that the fatty acids with shorter chains are to the values of previous lactation production correlated positively, while the longer chain are correlated negatively. Branched chain fatty acids arise by prolongation of chain that arose by oxidative deamination of branched amino acids **(Bauman *et al.,* 2006)**.

Monounsaturated fatty acids MUFA (26.26 ± 3.59%) have as the total sum the significant indirect relationship to indicators of total lactation production. Their content in milk fat decreased with the amount of total produced fat (-0.426), protein (-0.494), milk (-0.514), and with the increasing number of lactation days (r = -0.583, P <0.001). This relationship creates MUFALC (21.76 ± 3.65%), which is the essential component of MUFA. They have all negative coefficients in absolute value higher than the total MUFA, the closest is the correlation with the amount of milk (-0.640, P <0.001). Essential in this file is oleic acid C18: 1N9 OA (21.08 ± 3.59%), which one is also in an indirect relationship, more significant than the total MUFA, to the total milk production, with the highest correlation coefficient to the number of lactation days r = -0.645, P <0.001.

Table 2 Correlation coefficients of milk fat fatty acids content to milk production parameters in grazing dairy cows

n=134		**Days**	**Milk**	**Fat**	**Protein**
Fatty acid	**content**	**of lactation**	**total production (kg)**		
SAFA	x±Sx (%)	10–111	380–3735	14–155	15–109
C4:0	2.43±0.56	0.217	0.231	0.161	0.166
C6:0	2.06±0.51	0.412	0.430	0.347	0.379
C8:0	1.48±0.37	**0.512**	0.468	0.371	0.442
C10:0	**3.50±0.90**	**0.646**	**0.516**	0.398	**0.515**
C11:0	0.09±0.05	0.430	0.433	0.432	**0.513**
C12:0	**4.16±0.94**	**0.713**	0.499	0.367	**0.522**
C13:0	0.19±0.08	**0.518**	0.278	0.251	0.315
C14:0 MA	**12.71±1.55**	**0.592**	0.468	0.317	0.440
C15:0	1.40±0.24	0.223	-0.019	-0.068	0.065
C16:0 PA	**31.53±3.11**	0.264	0.359	0.396	0.397
C17:0	0.73±0.16	**-0.657**	**-0.642**	**-0.556**	**-0.651**
C18:0 SA	**7.98±1.66**	**-0.607**	-0.453	-0.326	**-0.509**
C20:0	0.11±0.03	-0.245	-0.400	-0.361	-0.432
C22:0	0.05±0.02	-0.303	-0.459	-0.382	-0.419
C24:0	0.03±0.02	-0.369	-0.403	-0.282	-0.352
BCFA					
C13:0i	0.16±0.05	**0.690**	0.395	0.291	0.469
C14:0i	0.19±0.05	0.254	-0.079	-0.191	-0.099
C15:0ai	0.61±0.13	0.055	-0.278	-0.357	-0.254
C16:0i	0.36±0.06	0.052	-0.208	-0.250	-0.266
C17:0i	0.41±0.08	-0.568	**-0.575**	**-0.516**	**-0.542**
C17:0ai	0.46±0.08	-0.520	**-0.599**	**-0.540**	**-0.588**
MUFA					
C10:1	0.38±0.12	**0.745**	**0.617**	0.479	**0.640**
C12:1	0.12±0.05	**0.712**	0.487	0.363	**0.562**
C14:1	1.13±0.27	**0.627**	0.364	0.267	0.441
C15:1	0.02±0.03	0.468	-0.109	-0.072	-0.105
C16:1n7cis POA	**1.49±0.3**	-0.213	-0.162	-0.102	-0.062
C16:1	0.43±0.06	-0.204	-0.355	-0.314	-0.244
C17:1n7cis	0.42±0.10	**-0.682**	**-0.531**	-0.398	**-0.508**
C18:1n9cis OA	**21.08±3.59**	**-0.645**	**-0.531**	-0.428	**-0.528**
C19:1	0.09±0.03	-0.210	-0.184	-0.194	-0.187
C20:1n9cis	0.15±0.06	-0.298	-0.320	-0.209	-0.306
PUFA					
C18:2n6cis,cis LA	**1.79±0.28**	-0.477	-0.316	-0.277	-0.295
C18:3n3cisALA	0.50±0.19	-0.242	-0.403	-0.469	-0.434
C18:2 9,11 CLA	**0.54±0.23**	-0.224	-0.404	-0.436	-0.403
C20:2n6	0.10±0.05	-0.417	-0.341	-0.286	-0.343
C20:3n6cis	0.04±0.02	-0.282	-0.264	-0.166	-0.185
C20:4n6cis ETA	0.12±0.05	-0.317	-0.234	-0.141	-0.195
C20:4n3cis	0.03±0.02	-0.189	-0.309	-0.252	-0.273
C20:5n3cisEPA	0.05±0.02	**-0.523**	-0.472	-0.377	-0.440
C22:5n3cisDPA	0.08±0.03	-0.254	**-0.512**	-0.498	-0.478

Legend: MA-myristic acid (A), PA- palmitic A, SA-stearic A, POA-palmitoleic A, OA-Oleic A, LA-linoleic A, ALAalfalinolenic A, CLA-conjugated linoleic A, ETA-eikosatetraenic A, EPA-eikosapentaenic A, DPA-dokosapentaenic A

Table 3 Correlation coefficients of milk fat fatty acids groups to milk production parameters in grazing dairy cows

Group of FAs	content x±Sx (%)	Days of lactation 10–111	Milk total production (kg) 380–3735	Fat 14–155	Protein 15–109
SAFASC	28.82±4.23	**0.657**	**0.509**	0.368	0.494
SAFAMC	33.49±2.94	0.196	0.289	0.339	0.330
SAFALC	8.17±1.69	**-0.607**	-0.459	-0.333	**-0.514**
SAFA	70.48±4.04	**0.599**	**0.537**	0.454	**0.517**
VFA	9.47±2.16	**0.507**	0.452	0.349	0.420
HCHFA	48.40±4.27	**0.584**	**0.528**	0.450	**0.544**
BCFA	2.02±0.33	-0.226	-0.487	**-0.512**	-0.480
MUFASC	2.16±0.53	**0.753**	0.466	0.344	**0.528**
MUFAMC	2.34±0.37	-0.384	-0.325	-0.235	-0.228
MUFALC	21.76±3.65	**-0.640**	**-0.538**	-0.443	**-0.536**
MUFA	26.26±3.59	**-0.583**	**-0.514**	-0.426	-0.494
PUFA	3.26±0.69	-0.474	-0.485	-0.468	-0.472
USFA	29.52±4.04	**-0.599**	**-0.537**	-0.454	**-0.517**
SCFA	30.98±4.52	**0.682**	**0.516**	0.374	**0.509**
MCFA	35.83±3.03	0.124	0.226	0.291	0.284
LCFA	33.19±5.32	**-0.674**	**-0.559**	-0.452	**-0.574**
n6	2.06±0.32	**-0.508**	-0.354	-0.295	-0.327
n3	0.65±0.24	-0.317	-0.490	**-0.523**	**-0.503**
n6/n3	3.46±1.02	-0.013	0.269	0.345	0.323
AI	3.01±0.63	**0.631**	**0.570**	0.470	**0.550**
EFA	2.96±0.61	-0.448	-0.451	-0.449	-0.445

Legend: SAFASC-Saturated fatty acids (FA) with short chain, SAFAMC-Saturated FA with middle long chain, SAFALC-Saturated FA with long chain, SAFA-Saturated FA , VFA- Volatile FA, HCHFA-Hypercholesterolaemic FA, BCFA Branched chain FA, MUFASC-Monounsaturated FA with short chain, MUFAMC-Monounsaturated FA with middle long chain, MUFALC-Monounsaturated FA with long chain, MUFA-Monounsaturated FA, PUFA-Polyunsaturated FA, USFA-Unsaturated FA, SCFA-Short chain FA, MCFA-Middle chain FA, LCFA-Long chain FA, AI-Atherogenic index, EFA-Essential FA

MUFASC (2.16 ± 0.53%) weaken the negative relationship of total MUFA, because they are with the total lactation production correlated positively, the closest with amount of protein (0.528) and the number of days (0.753, $P < 0.0001$). Especially in the first third of lactation are degraduated body fat reserves, from which comes part of fatty acids built in milk fat and gradually extends de novo biosynthesis of fatty acids with shorter chains. Higher organisms are able to incorporate double bonds in molecule by means of dehydrogenating enzyme system and reactions of chain growth **(Jenkins and McGuire, 2006)**.

Polyunsaturated fatty acids PUFA (3.26 ± 0.69%) identified in this work have in the carbon chain from 18 to 22 carbons. The whole group PUFA has to produced milk, fat, protein, as to the number of lactation days in indirect correlation almost the same coefficients

-0.468 – -0.485 (P <0.01). Even a group of essential fatty acids EFA has to produced milk, fat, protein, as to the number of days in lactation balanced indirect correlation coefficients -0.445 – -0.451 (P <0.01). In this trend is almost every polyunsaturated fatty acid of this group, the most significant is C18:2n6LA, with the highest content in milk fat (1.79 ± 0.28%), which has the strongest direct relationship to the number of days of lactation r = -0.477; P <0.01. The n6 acids group has significant relationship to the days of lactation (-0.508), the group of n3 acids to the amount of fat (-0.523). Their ratio n6/n3 shows the most significant rise in correlation with produced protein (0.323). In terms of human nutrition perhaps the most valuable conjugated linoleic acid CLA (0.54 ± 0.23%) with the number of days of lactation decreases statistically insignificant (r = -0.224), a significant decrease in its content is in relation to the amount of already produced milk (r = -0.404, P <0.01), fat (r = -0.436, P <0.01) and protein (r = -0.403, P <0.01). As we can see, change of relative share of PUFA to other fatty acids is mainly destined to advancing lactation, during which it decrease, indicating depletion of body fat stores of dairy cow. **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) and concluded that total production of fat and lactose plays probably an essential role in these relations.

The total amount of unsaturated fatty acids USFA (29.52 ± 4.04%) is primarily made up of long chain MUFALC (21.76%) and PUFA (3.26%), and their sum in our study was 25.02%. Only the rest, 4.50% is of MUFAMC (2.34%) and MUFASC (2.16%). It follows that USFA in the overall assessment act as long-chain. They are in quite close inverse relationship to the number of days of lactation r = -0.599, P <0.001, the total amount of produced milk (-0.537) and protein (-0.517).

Short chain fatty acids SCFA, middle chain fatty acids MCFA and long-chain LCFA cover the entire spectrum of milk fat. It is interesting that these parts of milk fat are each of them about a third of the fatty acids of milk fat, which offset each other for changes during lactation. SCFA (30.98 ± 4.52%) during the first third of lactation increased its share in milk fat statistically high significantly especially in correlation with the number of lactation days r = 0.682, P <0.0001, and the total quantity of milk r = 0.516, P <0.001. MCFA (35.83 ± 3.03%) was on the stage of lactation and milk production almost independent. Share of LCFA (33.19 ± 5.32%) was highly significantly decreasing by the rising number of days r = -0.674, P <0.0001, and the rising amount of produced protein r = -0.574, P <0.001. **Wathes *et al.* (2007)** developed mathematic models to determine how metabolic traits, milk yield and body condition score were interrelated. The comparison of differences between factors influencing the milk fat fatty acids composition is to be done with regard to stage of lactation of experimental animals.

CONCLUSION

In comparison studies of milk fat of cow groups with different treatment is important to take into account lactation stage and milk yield of individual cows in these groups. Cows within one herd show different milk fat FAs composition in dependence on milk yield and count of days after parturition. It may be related with origin and precursors of FAs in biosynthesis in mammary gland. Summarization of saturated, mono- and poly- unsaturated FAs without regard to carbon chain longness is not conducive for evaluation of results.

Grazing of dairy cows has a better value of the composition of milk fat from a health perspective, but at the account of lower production as seen by correlations at mountain farms with dairy grazing systems.

Acknowledgments: Contribution was based on project - APVV 0153-07. This article was written during realization of the project „CEGEZ no. 26220120042“, supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

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