FATTY ACIDS CONTENT AND COMPOSITION OF MILK FAT FROM COWS CONSUMING PASTURE AND TOTAL MIXED RATION

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Summary. The study was aimed to determine the composition and content of fatty acids in milk from cows consuming pasture and total mixed ratio (TMR). During this study it was established that the proportion of saturated fatty acids in total content of studied fatty acids was insignificantly lower in the milk fat from pasture fed cows (55.15%) than in the milk fat from confine fed cows (56.07%, $P > 0.05$). Statistically significant differences ($P < 0.05$) were observed in the content of unsaturated fatty acids (32.87 versus 30.07% for pasture and confine fed cows' milk fat, respectively). Pasture group cows produced significantly higher amounts of PUFAs – 6.02% versus 5.24% ($P < 0.05$) of TMR group. Within PUFAs, the highest concentration of C18:2 and C18:3 was observed in the milk fat from pasture cows (3.70% versus 3.27% and 0.51% versus 0.44%, $P < 0.05$, respectively).

The study showed that pasture fed cows produced significantly higher concentrations of CLA in milk fat compared to TMR fed cows – 0.66 and 0.51% ($P < 0.05$), respectively.

The study results revealed that milk fat from pasture fed cows had better proportion of unsaturated:saturated fatty acid in nutritional aspect, with more polyunsaturated FA and more CLA, than the milk fat from TMR fed cows had.

Key words: cows' milk fat, fatty acids, conjugated linoleic acid, pasture, TMR.

KARVIŲ, GANYTŲ IR ŠERTŲ VISŲ RACIONO PAŠARŲ MIŠINIU, PIENO RIEBALŲ RŪGŠČIŲ KOKYBINĖ IR KIEKYBINĖ SUDĖTIS

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Santrauka. Tirta dviejų bandų karvių pieno riebalų kokybinė ir kiekybinė sudėtis. Viena karvių banda buvo laikoma tvarte ir šeriama visų raciono pašarų mišiniu (VRPM), o kita banda – ganoma ganykloje ir papildomai gavo koncentratų.

Nustatyta, kad ganytų karvių pieno riebaluose sočiųjų riebalų rūgščių kiekis buvo nežymiai mažesnis nei karvių, šertų VRPM, t. y. 55,15 ir 56,07 proc. ($p > 0,05$). Nesočiosios riebalų rūgštys ganytų karvių pieno riebaluose sudarė 32,87 proc., o šertų VRPM – 30,07 proc. viso tirtų riebalų rūgščių kiekio ($p < 0,05$). Šertų VRPM karvių piene buvo 5,24 proc. polinesočiųjų rūgščių, iš jų 3,27 proc. sudarė C18:2 ir 0,44 proc. C18:3, o ganytų – atitinkamai 6,02, 3,70 ir 0,51 proc. ($p < 0,05$). Ganytų karvių bandos pieno riebaluose buvo 1,29 karto daugiau konjuguotos linolo rūgšties, t. y. 0,66 ir 0,51 proc., nei tvarte laikytų karvių ($p < 0,05$).

Be to, ganytų karvių pieno riebalų nesočiųjų ir sočiųjų riebalų rūgščių tarpusavio santykis mitybiniu požiūriu buvo geresnis.

Raktažodžiai: karvės, pieno riebalų rūgštys, konjuguota linolo rūgštis, ganykla, visų raciono pašarų mišinys.

Introduction. Researchers attending the Wisconsin Milk Board 1988 Milk Fat Roundtable indicated that the ideal nutritional milk fat would contain <10% polyunsaturated fatty acids (PUFA), <8% saturated fatty acids (SFA), and >82% monounsaturated fatty acids (MUFA) (Grummer, 1991). By modifying diets of lactating cows monounsaturated fatty acid (C18:1) content can be increased by 50 to 80% and may approach 50% of milk fatty acids by feeding lipids rich in 18-carbon fatty acids (Chouinard et al., 1998). Feeding low roughage diets increases the proportion of C 18:1 in milk fat and palmitic acid (C16:0) content in milk fat can be reduced by 20 to 40% (Gulati et al., 1997, Demeyer and Doreau, 1999).

Many researchers and commercial interests want to increase the levels of monounsaturated and polyunsaturated fatty acids (especially of omega-3 fatty acids and conjugated linoleic acid) in milk using different technological methods (Lock and Bauman, 2004). Polyunsaturated fatty acids are synthesized as an intermediate product in ruminal biohydrogenation (Griinari et al., 1999, Murphy et al., 2000, Laugalis et al., 2004). Much of recent research has focused on increasing the amount of conjugated linoleic acid (CLA) in milk fat because of the reported health benefits (Lee et al., 1994, Ip et al., 1999). CLA refers to various positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 octadecadienoic acid), each with conjugated double-bond arrangement. These bonds can occur in several positions such as 9 and 11, 10 and 12, and 11 and 13 (White et al., 2001).

Certain isomers of CLA, especially *cis*-9, *trans*-11 C18:2, have been identified as having anticarcinogenic and antiatherosclerotic properties (Lee et al., 1994, Ip et al., 1999). Some isomers of CLA have also been linked to enhanced growth of lean body mass (Park et al., 1999). Conjugated linoleic acid is found primarily in the meat and milk of ruminants. Milk fat is considered to be the richest natural source of CLA. CLA remains stable in processed dairy products (Lin et al., 1995) and the content in such products is a function of the concentration of CLA in raw milk. Naturally occurring CLA in a food form has biological activity and this activity is similar to that produced by mixture of CLA isomers delivered as free fatty acids (Ip et al., 1999).

The fatty acid composition of cow's milk, however, with the increase of animal productivity has become less favorable to human health in the last four decades due to the changes in feeding practices, higher proportion of concentrates and silage in diets with less grazing. Essential fatty acids and conjugated linoleic acid (CLA) concentrations have generally declined and with more "low fat" dairy products, human intake of this fatty acid has declined even more since ruminant food products are the main source of human CLA intake. Changing societal drivers and consumer demands require dairy products provided through more natural or sustainable production system. Researchers established, that milk from cows fed fresh green forage, especially those grazing grass, had a significantly higher unsaturated : saturated fatty acid proportion, with more polyunsaturated FA and more CLA, than the milk from silage fed cows had (Jahreis et al., 1997, Drackley et al., 2001, White et al., 2001, Bargo et al., 2006).

The aim of the research was to determine the composition and content of fatty acids in milk from cows consuming pasture and TMR.

Materials and methods. The experiment was conducted at the Research Center of Lithuanian Veterinary Academy, milk samples were collected daily during successive 6 days – from 26 May to 02 July 2006.

Milk samples from cows with two different feeding types – pasture and total mixed ration (TMR) – were analysed. The term "pasture raised" we define as the research system in which cows' nutrition is derived from grazing with supplement of concentrate.

The milk samples were taken from two dairy herds (farms) containing Black and White breeds, as well as their crossbreds with Holstein were tested.

Cows from the first (I) herd (n=130) were housed in a cowshed and had free stalls. All year round the cows were fed TMR *ad libitum* once a day. The yearly productivity of the examined herd was 6592 kg of milk (4.41% fat and 3.20% protein) in total. The mean value was calculated for a live weight of the cows 602±36.8 kg, age 4.8±1.17 years and day postpartum 146±64.4 at the beginning of the experiment. Daily productivity of the tested cow herd was 21.8±6.8 kg energy corrected milk (ECM) at the moment of investigation.

The daily feed ration consisted of (DM basis) 34% grasses silage, 24% maize silage, 3% barley brewers wet grain, 2% barley straw, 2% molasses, 34% compound concentrates, with mineral and vitamin supplements. Each cow received 17.7 kg DM. Nutrition composition of kg DM was: 7.0 MJ NEL, 15.0% crude protein, 4.5% crude fat and 21.6% crude fibre. The average intake of the *ad libitum* for each cow per day was calculated as the total intake of TMR divided by the number of cows. It was calculated once on the 3rd day of the experiment.

The composition and feeding value of compound concentrates for both herds is shown in Table 1. The cows were milked twice a day at 05.00 a.m. and 17.00 p.m. in a milking parlour.

Table 1. **Composition (% DM) and feeding value of concentrate compounds**

Ingredients	I herd	II herd
Wheat	15.0	20.0
Barley	12.0	19.0
Maize	15.0	16.0
Wheat bran	5.0	5.0
Maize gluten feed	6.0	6.0
Sugar beet pulp, dried	10.0	10.0
Sugar beet molasses	3.0	3.0
Brewers yeast, dried	3.0	1.0
Soya bean meal	10.0	3.0
Sunflower seed meal	5.0	5.0
Rape seed cake	10.0	5.0
Sunflower oil	1.0	1.0
Limestone ground	1.0	1.0
Phosphate defluorinated	0.5	0.5
Sodium chloride	0.5	0.5
Premix „Provimi"	3.0	3.0
Content 1 kg feed		
Dry matter, %	88.15	88.17
NEL, MJ	7.64	7.66
Crude protein, %	18.7	14.0
Crude fat, %	4.01	3.50
Crude fibre, %	7.1	7.2

Cows from the second (II) herd (n=120) were kept on pasture day and night in the summer time. The yearly productivity of the examined herd was 6701 kg of milk (4.44% fat and 3.28% protein) in total. Mean value was calculated for a live weight of the cows 614±33.4 kg, age 4.9±1.26 years and day postpartum 151±56.6 at the beginning of the experiment. Daily productivity of the tested cow herd was 20.3±5.3 kg ECM at the moment of investigation.

The cows were milked at 05.30 a.m. and 17.30 p.m. in a cowshed tied to stalls. After the process of milking the cows were individually fed by hand compound concentrates with mineral and vitamin supplements. The dosage of concentrates depended on cows productivity (for each kg ECM – 0.2 kg concentrates).

The botanical composition of pasture sward was (of total DM, %) as follows: 77.0 grasses (*Poa pratensis* L., *Festuca pratensis* Huds, *Phleum pratense* L., *Dactylis glomerata* L.), 10.9 legumes (*Trifolium repens* l., *Trifolium hybridum* L., *Lotus corniculatus* L.) and 12.1% forbs. The cows grazed in a poddock system. The poddock twice a day was divided into the parts using the fence of electric wire.

The pasture of the second grazing (18% DM/ kg) was at the stage of shooting grasses. The height of the herbage was 18 cm. The dry matter yield of grazed swards was 1.57 t/ha. The average daily pasture allowance was estimated by 12.5 kg for a dry matter per cow. They also consumed 3.6-5.4 kg of DM of compound concentrate. The daily feed ration consisted of (DM basis) 73.5% pasture grass and 26.5% compound concentrate. Each cow received 17.0 kg of DM. 1 kg of DM contained 6.71 MJ NEL, 15.2% crude protein, 3.99% crude fat and 18.6% crude fibre. The cows had free access to licking salt and fresh water.

Pregrazing herbage mass was estimated by cutting 10 strips (each 1x1 m, 4 cm stubble height) in a poddock before the cows were let into graze and the weight of the fresh herbage was recorded.

Post grazing herbage mass was estimated by cutting 10 strips next to early cut from the area last grazed by the cows. Herbage intake was estimated by taking the difference between pre and post grazing mass. At the same time the herbage samples were hand-plucked for the botanical and chemical analysis.

TMR and concentrates were sampled once on the 3rd day of the experiment. The nutrition value of the forage was determined on the basis of its chemical composition using standard indices and equations (H. Meyer, et al., 1999).

The nutritional value of the concentrated mixtures was calculated on the basis of tabular data on the strength of mixture composition (H. Meyer, et al., 1999). For the chemical analysis the forage samples were dried at 60 and 103^0C and were analysed for the concentration of dry matter, crude protein, crude fibre, crude fat and crude ash (Naumann C., Bassler R., 1993).

All milk fat samples were split into two portions for the analysis. One portion was refrigerated at 4 ºC and sent to the laboratory to be analyzed for fat, protein and lactose concentrations by infrared spectroscopy Lactoscope FTIR (FT 1.0. 2001; Delta Istruments, Holand). The data of milk composition are given in Table 2.

Table 2. **Chemical composition of tested cows milk**

Indices	I herd	II herd
Fat, %	4.0±0.11	4.14±0.04
Protein, %	3.11±0.01	3.33±0.02
Lactose, %	4.75±0.02	4.74±0.02

The results (table 2) indicated the concentrations of fat, protein and lactose. Milk results were similar for both herds and these parameters are characteristic for Black-and White cows, as well as for their crossbreds with Holstein breed.

As milk contains very low amounts of PUFA, in order to get more comprehensive results about fatty acid composition of butter collected milk samples (another portion) were tested.

Table 3. **Parameters of chromatographic analysis of fatty acids**

Parameter	Value
Column	BPX –70, capillary column length 120 m, ID 0.25 mm, film 0.25 µm (FAME)
Detector	FID
Temperature	
- column	60^{o}C for 3 min, 20^{o}C/min to 200^{o}C, 40 min at 250 ^{o}C
- injection	260 ^{o}C
- detector	270 ^{o}C
Nitrogen flow	3 ml/min
Injection	0.2 µl
Instrument	GC-17A Shimadzu

Identification and quantification of fatty acids were performed by gas chromatography employing capillary column and flame – ionization detection. The sample for fatty acid analysis was prepared according to the procedures described in EN ISO 5509:2000 and EN ISO 661:1989. The chromatographic peaks were identified on the basis of comparison with retention times of a mixture of referent material (EN ISO 5508:1990) obtained from Sigma-Aldrich from a local dealer. Parameters of chromatographic analysis are presented in Table 3.

We calculated the content of each relevant fatty acid as a percentage of the total fat in each sample.

The statistical analysis was performed using STATISTICA (Statistica for Windows, 1995) software. Statistical significance was declared at P < 0.05.

Results and discussion. The results of milk fatty acid composition and elementary statistical data on the groups of SFAs, MUFAs and PUFAs are given in Table 4. Fatty acid composition of milk fat differed in diet treatment.

Table 4. **Fatty acid composition of milk fat from pasture and TMR fed cows**

	Pasture				TMR			
	$\bar{x}$	S $\bar{x}$	min.	max.	$\bar{x}$	S $\bar{x}$	min.	max.
C4:0	2.39	0.59	1.86	2.71	2.47	0.32	1.04	2.96
C6:0	2.21	0.12	1.82	2.66	2.24	0.02	2.08	2.19
C8:0	1.50	0.03	1.32	1.86	1.46	0.02	1.30	1.64
C10:0	3.05	0.06	2.90	3.29	2.74	0.06	2.61	2.98
C12:0	3.22	0.05	2.96	3.49	2.83	0.09	2.63	3.29
C14:0	9.74	0.21	9.27	10.60	9.28	0.15	8.90	9.79
C14:1	0.90	0.04	0.82	1.07	0.95	0.05	0.84	1.18
C16:0	25.22	0.38	24.27	26.79	27.39	0.17	26.89	27.76
C16:1	1.90	0.04	1.81	2.03	2.15	0.04	2.07	2.39
C16:2	0.61	0.04	0.50	0.74	0.54	0.02	0.43	0.66
C16:3	0.36	0.03	0.24	0.47	0.35	0.03	0.30	0.48
C18:0	7.69	0.15	6.25	10.08	7.49	0.32	7.06	10.19
C18:1	23.70	0.22	22.90	24.10	21.41	0.76	20.51	25.46
C18:2	3.70	0.05	3.33	4.67	3.27	0.07	2.50	3.81
CLA[1]	0.66	0.06	0.45	0.89	0.51	0.04	0.27	0.59
C18:3	0.51	0.04	0.31	0.69	0.44	0.02	0.24	0.58
C20:0	0.13	0.16	0.10	0.16	0.17	0.08	0.12	0.23
C20:1	0.35	0.09	0.22	0.56	0.32	0.14	0.27	0.45
C20:2	0.04	0.18	0.01	0.05	0.05	0.07	0.03	0.07
C20:3	0.14	0.1	0.09	0.16	0.08	0.26	0.07	0.09
SFA	**55.15**	**0.27**	**50.75**	**61.64**	**56.07**	**0.31**	**52.63**	**60.3**
MUFA	**26.85**	**0.24**	**25.75**	**27.76**	**24.83**	**0.33**	**23.69**	**29.48**
PUFA	**6.02**	**0.49**	**4.93**	**7.67**	**5.24**	**0.59**	**3.84**	**6.28**

[1] – *cis*-9, *trans*-11 18:2 isomer only

Means of short chain fatty acids C4:0 were 2.39 and 2.47, C6:0 - 2.21 and 2.24 % and C8:0 - 1.50 and 1.46 % of pasture and TMR feeding type, respectively. It is higher than reported in studies (Liutkevičius et al., 2001, White et al., 2001). Pasture fed group produced significantly (P<0.05) more C10:0, C12:0, C14:0, C18:1, C18:2 and C18:3 fatty acids. TMR cows produced significantly (P<0.05) more C14:1, C16:0, C16:1. In the total content of studied fatty acids the proportion of saturated fatty acids was insignificantly lower in the milk fat from pasture fed cows (55.15 %) than in the milk fat from TMR fed cows (56.07 %). The lower proportion of milk fat SFAs seems to be favourable for human health because of their negative role in arteriosclerosis (Pfeuffer and Schrezenmeir, 2000). Taking into consideration the previous research results the significant (P < 0.05) differences in proportion of unsaturated fatty acids (32.87 versus 30.07 % for pasture and TMR fed cows' milk fat, respectively) were observed and comparing with literature data (Timmen and Patton, 1988, Jahreis et al., 1997, Kelly et al., 1998, Drackley et al., 2001, White et al., 2001). The highest respective proportion of all PUFAs (especially C18:2 and C18:3) in the milk fat was detected in pasture grazing cows (P < 0.05).

Some of the fatty acid levels showed different results in comparison to previous work done on pasture and TMR based systems. For example, there were statistically significant differences between the groups in the total production of short- and medium-chain fatty acids (C10:0 to C14:0). Dhiman et al. (1999) did not report any differences in the total amount of short- and medium-chain fatty acids, but Timmen and Patton (1988) and Kelly et al. (1998) reported the total content to be lower in the pasture fed cows. Jahreis et al. (1997) Timmen and Patton (1988) reported that pasture consumption increases the production of C 18:1. Our study confirmed these results – the highest level of C 18:1 was obtained in pasture group.

Proportion of butyric acid (C4:0) was similar in both feeding types (2.39 and 2.47%), and it is higher if compared to literature data (Dhiman et al., 1999, White et al., 2001). Components from the SFA group, especially the capric (C10:0) and lauric acids (C12:0), were found in high abundance in milk from both (pasture and TMR fed) cow herds.. Statistically significant differences in the proportion of saturated acids between both feeding types were determined for C10:0, C12:0 (3.05 and 2.74%, 3.22 and 2.83% in pasture and TMR, respectively, $P < 0.05$). Significantly higher content of palmitic acid (C16:0) was found in TMR fed cows (27.39 and 25.22% TMR and pasture, respectively, $P < 0.05$). Our values of the above mentioned fatty acids are close to those reported by Liutkevičius et al. (2001), White et al. (2001).

We revealed that pasture cows produced 29% higher concentration of CLA than the TMR cows (0.66 vs. 0.51%, $P < 0.05$). Other studies with pasture fed cows with grain or silage supplementation reported near the same levels of CLA (Timmen and Patton, 1988, Kelly et al., 1998).

CLA is formed as intermediate product in the digestion of dietary fat in the rumen (Abu-Ghazaleh et al., 2002). It is the product that is formed as an intermediate during the biohydrogenation of linoleic acid (C18:2) to stearic acid (C18:0) by *Butyrivibrio fibrisolvens*. Pasture feeding increases CLA of milk, especially with grass at early growth stage (Chouinard et al., 1998, Chilliard et al., 2000). The high C18:3 content of young grass and low fibre content most probably interact together in order to increase the production of CLA or its *trans* C18:1 precursors. Our study determinated high levels of C18:3 fatty acid. It can lead to the formation of higher levels of CLA under certain conditions by modifying rumen biohydrogenation (Lock and Garnsworthy, 2002). Ruminal bacteria, which synthesize long-chain fatty acids from odd-numbered vaccenic acid, are major source of those odd-numbered chain fatty acids in milk fat. How bacteria regulate the lipid composition is still unclear; however, it is well established that dietary lipids can alter the lipid composition of rumen bacteria. The values of mentioned fatty acids (C18:1 and C18:3) are much higher than reported by Jahreis et al. (1997), Liutkevičius et al. (2001) and White et al. (2001).

Kelly et al. (1998) reported that the amount of CLA produced more variations in the grazing cows than in the confinement group. Jahreis et al. (1997) reported that the lowest variation of CLA was detected in milk samples of the confinement fed cows. Data of our study show little daily variation within the pasture fed cows. There were no significant variations in the confinement fed group

Conclusions. During this study it was established that the proportion of saturated fatty acids was insignificantly lower in the milk fat from pasture fed cows (55.15%) than in the milk fat from confine fed cows (56.07%) in total content of studied fatty acids. Statistically significant differences ($P < 0.05$) were observed in the content of unsaturated fatty acids (32.87 versus 30.07% for pasture and confine fed cows' milk fat, respectively). Pasture group cows produced significantly higher amounts of PUFAs – 6.02% versus 5.24% ($P < 0.05$) of TMR group. All PUFAs, especially C18:2 and C18:3 showed the highest respective content in the milk fat of pasture cows (3.70% versus 3.27% and 0.51% versus 0.44%, $P < 0.05$, respectively).

The study showed that pasture fed cows produced significantly higher concentrations of CLA in milk fat compared to TMR fed cows – 0.66 and 0.51% ($P < 0.05$), respectively.

The milk fat from pasture fed cows had a higher unsaturated : saturated fatty acid proportion, with more polyunsaturated FA and CLA, than the milk fat from TMR.

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