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VPLYV FLORISTICKÉHO ZLOŽENIA PASIENKOVÉHO PORASTU NA KVALITU OVČIEHO MLIEKA

INFLUENCE OF FLORISTIC GRAZING COVER ON SHEEP MILK QUALITY

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Changes in the fatty acids (FA) composition of pasture plant species during pasture season in two meteorologically different years and their changes in ewes' milk were analyzed by gas chromatography. The changes in *cis*-9, *trans*-11 18 : 2 (CLA) content are determined primarily by the α-linolenic acid (ALA) content in pasture plants. The content of ALA as the most abundant FA in pasture, in dryer year 2007 decreased from mid-May to mid-August from 62.4% to 38.8% (g 100 g^{-1} plant fat content) ($P < 0.001$) and that in mid-September increased to a similar value as that in May. A similar trend was also found in CLA content in milk, when 2.3% content in May decreased to 1.3% ($P < 0.001$) content in July and subsequently it increased to 2.3% content in September (g 100 g^{-1} milk fat content). In the year 2008, which was colder and rainier during pasture season, the highest CLA contents were noted in May (2.3%) and September (2.0%), nevertheless, a minimum in milk CLA content (1.3%) was found in June linked with a higher average temperature and lesser rainfall compared to July 2008. According to the FA composition of ewes' milk fat, including CLA content, the September ewes' milk was similar in quality to the May milk.

Key words: fatty acid composition, pasture plants, ewes' milk, seasonal changes

The quality of grassland is assessed by the production of plant dry matter, weight gain and milk production of heifer, dairy cows and small polygastric animals. In addition to these economic aspects, there are also significant health concerns of milk composition of animals fed on pasture. Saturated FA C4-C10 (mainly butyric acid), monounsaturated (mainly oleic acid) and polyunsaturated acids (mainly n-3 acids) are considered to be potentially beneficial to health. Saturated FA C12-C16 and some *trans*-acids are dangerous in regard to cardiovascular diseases. It is therefore important to quantify changes in forage content of precursors of FA in milk fat to determine the modifiers of positive health affecting FA content in dairy products. An ongoing research is focused especially on conjugated octadecadienoic acid (CLA) isomers, mainly *cis*-9, *trans*-11 CLA isomer, showing potential anticarcinogenic, antiatherogenic, antidiabetic, antiobesity and immunomodulatory functions. Dutch authors (Elgersma et al., 2006) stated that in the past four decades, the FA composition of milk became less appropriate for human health. This is due to the changes in feeding and management animals given the higher proportion of concentrates and silage ratio and lesser forage ratio. They showed that concentrations of essential FA (α-linolenic acid ALA and linoleic acid LA), as well as CLA in dairy products decreased significantly; with CLA content decreased most significantly by 300% (from 1.5% to 0.5% of total fat content).

Milk from ruminants fed with pasture have a higher ratio of unsaturated to saturated FA, more polyunsaturated FA including CLA compared with milk of ruminants fed with silage and concentrates. The study of the relationship between the FA content in pasture consumed by ruminants and their milk fat composition found that the presence of CLA in milk fat of ruminants resulted from isomerization and biohydrogenation of ALA and LA being the major unsaturated FA in grassland by rumen bacteria and Δ-9 desaturase activity in the mammary gland (Collomb et al., 2006). Dhiman et al., (2000) suggested that the ALA content in the feed increased the CLA content in cow milk, when the content ratio of LA/ALA in the feed was 1 : 1 or less.

Modification of FA composition of milk is only minimally affected by the stage and order of lactation and breed. The most significant factor affecting of FA composition and mainly the CLA content in milk is the animal diet. The content of CLA in milk fat may be modified by the feed containing different content of fat precursors. The content of ALA and LA precursors in the feed depends on plant species in pasture, degree of vegetation, temperature and light intensity (Hawke, 1973). Dewhurst and King (1998) found, that fresh grass had higher contents of ALA and LA than lifted grass and hay grass. Under greenhouse conditions Clapham et al. (2005) found, that the content of FA including ALA and LA decreased with the mature degree of plants at half to the one third of the content. Grasses, however, retained a similar composition of FA, when the content of ALA and LA was on average 66% and 13% of total content of FA, respectively. Dhiman et al. (1999) found that milk of cows grazing on pasture without added grain or concentrates contained 500% more CLA than that of cows fed with grain and concentrates. These results show that grazing is the best natural way to increase CLA content in milk and also meat in ruminants. The higher CLA content in milk of ruminants fed on young pasture is explained by the fact that fresh grass promotes the synthesis of CLA by increased activity of Δ-9 desaturase in mammary gland and by contribution of other yet unknown factors (Lock and Garnsworthy 2003).

We studied the effect of forage on FA profile in milk during transition from dry winter to natural pasture diet also in relation to lower availability of forage in a previous work (Ostrovský et al., 2009). The aim of this work was to analyze by gas chromatography the changes in composition of the FA samples

of pasture during pasture season April – September in meteorologically different years 2007 and 2008 as well as equivalent changes in FA composition in ewes' milk. Based on these results, to propose possible improvements in the FA composition of grasslands pasture by oversowing grass and legume plants with a higher content of ALA precursor to obtain more health profitable composition of milk fat in terms of CLA content and other FA.

Material and methods

The floristical groups and main plant species of pasture during pasture season April – September 2007 and 2008 were determined at the experimental farm of Research Institute of Animal Production in Trenčianska Teplá using a method of projective dominance (Regal and Krajčovič, 1963) once a month except for July 2007. In 2008 only average pasture samples were sampled monthly. The plant pasture samples from five area sites (500 x 500 mm) were homogenized and the proportion of floristical groups (grasses, legumes and herbs) as well as plant species within these groups were analyzed. Average plant samples as well as three dominant plant species of floristical groups in individual months of grazing were analyzed by gas chromatography for the content of individual FA methyl esters (Meľuchová et al., 2008). The results of floristical analysis of plant samples were not analyzed statistically since only average samples were available. The herbage sample fat content was determined by Soxhlet method (1998/64/ES).

In 2008 oversowing was performed at two experimental sites in Trenčianska Teplá using a common mixture of six grass species: *Dactylis glomerata* cv. NIVA (9.4%), *Festuca rubra* cv. FEROTA (15.6%), *Festuca pratensis* cv. LEVOČSKÁ (18.8%), *Poa pratensis* cv. BALIN (6.3%), *Lolium perenne* cv. TARPAN (15.6%), *Lolium hybridum* cv. ODRA (18.8%) and one legume species *Trifolium repens* cv. RIVENDEL (15.6%) (g 100 g^{-1} total oversowing), weight sowings was 32 $kg.ha^{-1}$. Oversowing was accomplished by disk drill marks VREDO.

The experimental farm in Trenčianska Teplá kept 350 dairy ewes of three breeds (improved Valachian, Tsigai, Lacaune) and their crosses. During the pasture season from mid-April to mid-September the ewes were grazed on natural pasture, and concentrate 0.2 kg per day was added to feed during machine milking. The bulk samples of ewes' milk for gas chromatographic analysis of FA were taken twice a month, and more freque ntly at the beginning of the pasture season. Meteorological conditions, the average air temperature (°C) and average rainfall (mm) during pasture season in Trenčianska Teplá in 2007 and 2008 are shown in Table 1.

The FA contents of plant and milk samples were analyzed as follows (Meľuchová et al., 2008). The lipids of plant and milk samples were extracted using chloroform-methanol mixture (2 : 1), the extracts were filtered through anhydrous sodium sulfate, and then dried and stored at -18 °C. For the preparation of FA methyl esters, the base-catalyzed methylation procedure with a sodium methoxide solution in methanol was used. Gas chromatographic analyses of plant and milk extracts were performed on a gas chromatograph Agilent Technologies 6890N (Agilent, Waldbronn, Germany) with flame ionization detector and a 5973 Network mass-selective detector. The FA methyl esters were separated in a capillary column 60 m x 0.25 mm x 0.25 μm coated with DB-23 stationary phase (J&W Scientific, Agilent Technologies, USA) by programmed column temperature of 70–240 °C. For GC resolution of *trans*-11 from *trans*-10 18 : 1 isomer a column of 100 m x 0.25 mm i.d. x 0.2 μm film thickness of CP-Sil 88 stationary phase (Varian, Palo Alto, CA, USA) and isothermic temperature of 150 °C was used. FA separation in this column was also used for chemometrical deconvolution of gas chromatographic unresolved CLA isomers triplet including *cis*-9, *trans*-11 18 : 2 isomer (Blaško et al., 2009). Separated components were identified by reference materials, published retention data and mass spectrometry measurements. The chromatograms were evaluated quantitatively using a method of internal normalization and published response factors for flame ionization detector for FA methyl esters (Ackman, 2002).

Statistical analysis of determined content of FA compounds in plant and milk samples was carried out from three parallel determinations. One-way ANOVA statistical package was applied to evaluate the significance of differences between the FA contents within season and between 2007 and 2008 seasons. Significant differences were considered at the level $P < 0.05$.

Table 1 Average daily air temperature in °C and average daily rainfall in mm during pasture season in Trenčianska Teplá in 2007 and 2008

	Average daily temperature in °C (1)		Average daily rainfall pattern in mm (2)	
	2007	2008	2007	2008
April	11.0	10.0	0.1	1.5
May	16.4	14.8	3.0	1.8
June	18.6	19.0	3.5	2.6
July	19.4	19.8	2.3	3.5
August	19.4	18.3	2.5	2.6
Average (3)	17.0	16.3	2.29	2.4

Tabuľka 1 Priemerná denná teplota vzduchu v °C a priemerný denný úhrn zrážok v mm počas pasienkovej sezóny v Trenčianskej Teplej v rokoch 2007 a 2008
(1) priemerná denná teplota, (2) priemerný denný úhrn zrážok, (3) priemer

Results and discussion

Seasonal changes in the composition of fatty acids in plants of pasture

The pasture of experimental farm in Trenčianska Teplá is characterized by less availability of forage vegetation in summer pasture period (Ostrovský et al., 2009), therefore meteorological conditions can affect the composition of pasture forage as well as FA composition of milk of ewes fed on pasture. Established changes in the composition of floristical species and the three dominant plant species as % of total amount as well as fat (g 100 g^{-1} dry plant matter samples) during the ewes' pasture season are presented in Table 2. This table shows significant changes in the composition of floristical species and plant species during the pasture season. The grasses had the highest content in pasture throughout the whole season, with their content being increased from 50% to 82% from April to August, and later decreased to 64% in September. The content of legumes in forage increased from

Table 2 Changes in the floristical groups and the three dominant plant species (% of total amount) and fat (g 100 g^{-1} dry plant samples) during the ewes' pasture season in Trenčianska Teplá

Sampling date (1)	Floristical groups (2)		Plant species (3)		
			Bromus inermis	*Trifolium repens*	*Achillea millefolium*
19/04/07	Grasses (4) Leguminous plants (5) Herbs (6)	50 20 30	28	20	15
	Fat (7)	3.4	3.2	3.3	3.1
			Bromus inermis	*Trifolium repens*	*Achillea millefolium*
16/05/07	Grasses (4) Leguminous plants (5) Herbs (6)	62 27 11	40	25	12
	Fat (7)	3.5	2.6	3.6	4.2
			Festuca rubra	*Trifolium repens*	*Achillea millefolium*
15/06/07	Grasses (4) Leguminous plants (5) Herbs (6)	68 18 14	42	17	10
	Fat (7)	3.8	3.4	4.2	5.5
			Festuca rubra	*Trifolium repens*	*Achillea millefolium*
21/08/07	Grasses (4) Leguminous plants (5) Herbs (6)	82 7 11	45	6	6
	Fat (7)	3.2	3.4	3.3	4.2
			Dactylis glomerata	*Trifolium repens*	*Achillea millefolium*
12/09/07	Grasses (4) Leguminous plants (5) Herbs (6)	64 25 11	31	18	7
	Fat (7)	4.2	4.4	4.1	5.2

Tabuľka 2 Zmeny v floristických skupinách a tri dominantné druhy rastlín (% z celkovej masy) a tuk v g 100 g^{-1} sušiny počas pasienkovej sezóny v Trenčianskej Teplej
(1) dátum odberu, (2) floristická skupina, (3) druhy rastlín, (4) trávy, (5) leguminózy, (6) byliny, (7) tuk

20% to 27% in the period from April to May, decreased from 18% to 7% in the period from June to August, and finally it increased to 25% in September. The content of herbs in pasture decreased from 30% in April to 11% in September.

There were 27 plant species identified in pasture during the pasture season. The portion of the three dominant plant species in different months is shown in Table 2. *Bromus inermis* LEYSS. (28–40%) was the most abundant species in pasture during the period April – May, whereas *Festuca rubra* L. (42–45%) and *Dactylis glomerata* L. (31%) prevailed in June to August and September, respectively. *Trifolium repens* L. is another important plant which 20–25% portion in May – April decreased to 6% in August, and then increased to 18% in September (similar to that in April).

The fat content in average plant samples increased from 3.4% in April to 3.8% in June, decreased to 3.2% in August, and increased to 4.2% in September. The fat content in individual plants changed with different seasons, i.e. that in *Bromus inermis* LEYSS. increased from 3.4% in August to 4.4% in September, that in *Trifolium repens* L. increased from 3.3% to 4.2% in the period April – June, then decreased to 3.3% in August, and eventually increased to 4.1% in September. The fat content in *Achillea millefolium* L. increased from 3.1% in April to 5.5% in June, decreased to 4.2% in August, and increased to 5.2% in September (similar to that in June). All the three plant species showed increases in fat content from April to June, decreases in August, and significant increases in September to degrees comparable with the highest fat contents throughout the pasture season.

The changes in the FA composition of pasture plant samples and three dominant plant species (in g 100 g^{-1} plant fat) during pasture season in Trenčianska Teplá in 2007 are presented in Table 3. The data show that pasture included four major FA: ALA, LA, palmitic (PA) and oleic (OA) acid, their total content was on average 90%. The contents of these FA and their content ratio in pasture and plants changed during the pasture season. The changes were evaluated using one-way ANOVA being FA content in average pasture sample and month dependent and an independent variate, respectively. ALA was the most abundant FA in the average plant sample, which content decreased from 62% to 39% ($P < 0.001$) from May to August and increased to 60% in September to a similar content ($P > 0.05$) as that the beginning of the pasture season. Linoleic acid, the second CLA precursor showed reverse changes in pasture content during the pasture season compared to ALA. The LA content in pasture increased from 14% in May to 20% in August ($P < 0.001$), and then decreased to 17% in September ($P < 0.01$). The PA content in pasture increased from 13% in April to 19% in June ($P < 0.001$), and then decreased to 13% in September, thus similarly to that in April. The OA content increased from 1.3% in May to 8.8% in August ($P < 0.001$), and decreased to 1.8% in September (similarly to that at the beginning of the season).

Table 3 FA composition of pasture plant samples and three dominant plant species in g 100 g^{-1} plant fat during pasture season in Trenčianska Teplá in 2007

Sampling date (1)	19/04/07				16/05/07				15/06/07				21/08/07				12/09/07			
FA in g 100 g^{-1} (2)	APS	BI	TR	AM	APS	BI	TR	AM	APS	BI	TR	AM	APS	BI	TR	AM	APS	BI	TR	AM
10 : 0	–	–	–	–	–	–	–	0.1	0.1	0.3	–	–	0.1	–	0.1	–	–	–	–	0.1
12 : 0	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.5	0.8	0.2	0.1	0.6	0.5	0.3	0.3	0.2	0.1	0.1	0.1
14 : 0	0.8	0.5	0.9	0.9	0.4	0.4	0.4	0.3	1.0	1.7	0.7	0.7	1.8	1.0	0.4	1.7	0.4	0.3	0.3	0.3
15 : 0	0.2	0.1	0.3	0.2	0.2	0.1	0.3	0.2	0.3	0.5	0.3	0.4	0.5	0.5	0.3	0.3	0.2	0.1	0.2	0.1
16 : 0 PA	[a]13.3	12.0	12.2	14.3	[a]13.5	12.6	11.9	14.0	19.3	23.1	18.4	18.0	16.8	15.3	14.0	16.3	[a]13.1	11.7	11.4	11.2
16 : 1	2.0	1.9	2.1	1.9	2.2	2.0	2.4	2.3	0.4	0.4	0.3	0.5	1.7	1.9	2.2	1.9	2.1	2.0	2.0	1.6
17 : 0	0.7	0.5	0.9	0.6	0.1	0.2	0.2	0.2	0.4	0.6	0.5	0.4	2.1	0.5	0.4	2.3	0.2	0.2	0.2	0.1
18 : 0	1.8	1.1	2.7	1.5	1.6	1.0	1.9	1.0	2.7	2.8	1.7	3.0	3.8	1.7	2.6	2.9	1.4	1.2	1.3	1.1
9*t* 18 : 1	0.2	0.3	0.2	0.2	0.2	0.4	0.2	0.2	0.6	1.1	0.6	0.7	0.3	0.2	0.2	0.3	0.1	0.1	0.1	0.1
9*c* 18 : 1 OA	[a]2.1	2.1	3.1	2.4	1.3	1.7	1.5	1.3	3.5	4.8	2.5	2.8	8.8	4.9	3.3	5.2	[a]1.8	1.5	1.7	1.4
18 : 2 LA	[a]14.3	13.1	13.8	19.5	[a]14.3	12.8	14.4	20.6	[b]19.1	20.5	20.4	23.7	[b]19.7	24.9	18.1	17.8	16.7	16.0	16.5	19.0
18 : 3 ALA	[a]60.1	62.9	59.6	53.9	[a]62.4	63.7	62.4	56.8	45.8	35.7	48.1	47.5	38.8	42.3	48.7	44.0	[a]59.5	62.9	63.2	62.2
20 : 0	0.4	0.6	0.6	0.5	0.6	0.9	0.6	0.1	2.1	4.0	1.6	0.4	1.1	1.7	0.6	0.6	0.6	0.6	0.4	0.1
22 : 0	0.7	1.1	0.5	0.4	0.9	1.3	0.5	0.5	1.5	1.4	0.9	0.8	1.0	1.8	0.6	0.6	0.6	0.4	0.4	0.3
24 : 0	0.5	0.4	0.7	0.6	0.6	0.4	0.7	0.7	1.6	0.9	1.3	1.2	0.5	0.5	0.8	0.5	0.5	0.5	0.6	0.5

Note: FA – fatty acids, APS – Average pasture sample, BI – Bromus inermis, TR – Trifolium repens, AM – Achillea millefolium, FR – Festuca rubra, DG – Dactylis glomerata, TO – Taraxacum oficinale, ALA – α-linolenic acid, LA – linoleic acid, OA – oleic acid, PA – palmitic acid
A one-way ANOVA statistical package was applied to evaluate the significance of differences between the FA contents. Significant differences were considered on the level P<0.05. The differences between means assigned with the same letter are not statistically significant.
Poznámka: FA – mastné kyseliny, APS – priemerná vzorka pastviny, BI – Bromus inermis, TR – Trifolium repens, AM – Achillea millefolium, FR – Festuca rubra, DG – Dactylis glomerata, TO – Taraxacum oficinale, ALA – α-kyselina linolénová, LA – kyselina linolová, OA – kyselina olejová, PA – kyselina palmitová
Na vyhodnotenie významu rozdielov medzi obsahmi mastných kyselín sa použil štatistický balíček ANOVA. Významné rozdiely sa vzali do úvahy na úrovni P < 0,05. Rozdiely medzi priemermi v rámci toho istého písmena nie sú štatisticky významné.

Tabuľka 3 Zloženie mastných kyselín vo vzorkách pasienkových rastlín a tri dominantné druhy rastlín v g 100 g^{-1} rastlinného tuku počas pasienkovej sezóny v Trenčianskej Teplej v roku 2007
(1) dátum odberu, (2) mastné kyseliny

Similar seasonal changes in FA content were found in dominant plant species. These changes were most significant in *Trifolium repens* L. belonging to three dominant plant species of pasture throughout the pasture season. The content of most abundant ALA decreased from 61% in April – May to 48% in June – August, and increased to 63% in September (similarly to that at the beginning of the season). The LA content increased from 14% in April – May to 19% in June – August, and decreased to 17% in September. The PA content increased from 12% in April – May to 18% in June, and it decreased to 14% in August and 11% in September (similarly to that in April). In *Achillea millefolium* L. the ALA content decreased from 55% in April – May to 48% in June, the LA content increased from 20% in April – May to 24% in June, and the PA content increased from 14% in April – May to 18% in June. Even when FA content changes were compared for the remaining pasture plant species (*Bromus inermis* LEYSS. and *Festuca rubra* L.), there was a general observation that the ALA content in plants decreased in April – August and then increased in September to a similar value as that at the season beginning. The LA content in plants increased in April – August and then decreased in September. The PA content in plants increased in April – June and then decreased in September. The OA content in plants increased from April to August, and then decreased in September to a similar value as that in April. Thus, in contrast with data published by Clapham et al. (2005) suggesting relatively time-unchanged composition of the FA in studied plants in greenhouse conditions, we noted the changes in fractional contribution of individual FA to total acid content under our pasture conditions.

Table 3 shows that the FA composition in ewes' pasture season 2007 in Trenčianska Teplá changed most significantly for the most abundant FA, i.e. ALA and LA, which are the precursors for CLA and *trans*-vaccenic acid (TVA) in milk of ruminants. The role of TVA lies in being the precursor for the synthesis of *cis*-9, *trans*-11 CLA in ruminant mammary gland and other tissues (Collomb et al., 2006).

The seasonal changes in the composition of FA in pasture plants in 2008 as average content of three experimental sites in Trenčianska Teplá are presented in Table 4. ANOVA analysis was similar as mentioned in Table 3. The comparison of FA composition of pasture in 2007 and 2008 (Table 3 and Table 4) show that main difference lies in the content of ALA, which in pasture season 2008 was only less changed ($P < 0.05$) (average content 51.2%) compared to 2007 ($P < 0.001$), and the ALA contents in May (57.2%) and September (50.4%) were lower ($P < 0.05$) and in June – August higher (51.9%) than those in 2007 ($P < 0.01$). A decrease of ALA content was accompanied by increase of LA content similar as in year 2007.

Seasonal changes in the composition of fatty acids in ewes' milk

The changes in the composition of most abundant FA in pasture samples and the corresponding changes in the composition of significant FA in ewes' milk during pasture season in Trenčianska Teplá are shown in Figure 1. The

Figure 1 Changes in fatty acids (FA) composition of pasture (doted lines) and in ewes' milk fat (full lines) during pasture season in Trenčianska Teplá in 2007 in g 100 g^{-1}
cis-9, *trans*-11 18 : 2, CLA; trans vaccenic acid, TVA; α-linolenic acid, ALA; linoleic acid, LA; oleic acid, OA; palmitic acid, PA

Obrázok 1 Zmeny v zložení mastných kyselín (FA) vplyvom rastlinných druhov (prerušovaná čiara) a obsah tuku v ovčom mlieku (plné čiary) v pasienkovej sezóne v Trenčianskej Teplej v roku 2007 v g 100 g^{-1}
cis-9, *trans*-11 18 : 2, CLA, trans vakcénová kyselina, TVA, α -linolénová kyseliny, ALA, kyselina linolová, LA, kyselina olejová, OA, kyselina palmitová, PA
(1) obsah mastných kyselín

changes in FA composition of ewes' milk during pasture season in the years 2007 and 2008 are found in Table 5. The significance of differences between FA content was evaluated within the seasons being month an independent variate. To evaluate the differences between seasons 2007 and 2008, FA contents determined in the same month were compared. It is evident that the CLA milk content in 2007 was highest at the beginning of pasture season (2.3%) and similar with the highest published CLA contents in milk of grazing ewes and cows (Soják et al., 2009). Then, it decreased to 1.3% ($P < 0.001$) in July, and subsequently increased to 2.3% in mid- September similarly to that at the season beginning. The changes in CLA content in milk correspond to those in ALA content in plant samples during the pasture season with highest contents (60%) in pasture both at the beginning and the end of pasture season, and lowest contents (39%) in August. A seasonal 35% decrease in ALA content in pasture is equivalent to a 45% decrease in CLA content in milk.

Nudda et al. (2005) reported a monotonic decrease in CLA content (as well as in ALA content) in ewes' milk from 2.2% to 1.1% during the pasture season (second half of February – the first half of June). We found a repeated increase in CLA content in September milk (similarly to May milk) upon a decrease in the period May – July. This is consistent with the higher ALA content in September pasture. Our data suggest that ewes' milk and related products ("bryndza") made in September have a similar quality to those made in May with respect to FA composition of ewes' milk fat including CLA content.

A characteristic decrease of CLA content in milk and ALA content in pasture during the summer pasture season was related to meteorological conditions at Trenčianska Teplá in 2007 (very warm and below-average rainfalls, Table 1). Compared with the year 2007, the meteorological conditions in 2008 were characterized by lower average daily temperatures and higher rainfall during the pasture season reflected in higher average relative air humidity (72.4% and 69.7%, respectively). A meteorological favourable year 2008 displayed more stable FA composition of forage during the pasture season than 2007, including higher ALA/LA ratio in summer months (2.4 and 2.0, respectively) and lower ALA/LA ratio in May and in September (3.1 and 4.0, respectively) (Table 3 and 4). The LA content changed most being higher on average by 2.2% (12% relatively). The changes in TVA/CLA content ratio and *trans*-11/*trans*-10 18 : 1 ratio during pasture season in 2007 as well as 2008 years were found ($P < 0.001$). The *trans*-10 18 : 1 content was low in all ewes' milk samples (still higher in 2008). In contrast to TVA the *trans*-10 18 : 1 can not serve as substrate for synthesis of CLA through Δ-9 desaturation and was positively related to the degree of coronary artery disease in patients (Hodgson et al., 1996).

Figure 2 shows relationships between the CLA content in milk and the ALA content in pasture in Trenčianska Teplá based on measurements in 2007 and 2008. It is clear that the CLA content in milk varied with months of the pasture season similarly for both years. However, the lowest CLA content in milk (1.3%) was found in June 2008 and in July 2007, in association with a higher average temperature in June 2008

Table 4 Changes in the FA composition of plant pasture samples during pasture season in Trenčianska Teplá in 2008

FA in g 100 g^{-1} (2)	Sampling date (1)				
	14/05/08	16/06/08	09/07/08	18/08/08	17/09/08
10 : 0	0.02	0.02	0.02	0.03	0.03
12 : 0	0.1	0.2	0.4	0.3	0.4
14 : 0	0.4	0.5	0.7	1.2	0.9
15 : 0	0,1	0,2	0,2	0,3	0,2
16 : 0 PA	^{a}15.8	^{a}17.0	^{a}16.7	^{a}15.7	^{a}15.3
16 : 1	2.0	1.8	1.8	2.0	1.8
17 : 0	0,2	0,3	0,3	0,3	0,7
18 : 0	1.5	1.8	1.9	2.4	2.3
9*t* 18 : 1	0.2	0.3	0.1	0.5	0.3
9*c* 18 : 1 OA	^{a}2.8	^{a}3.0	^{b}3.8	^{b}3.9	4.9
18 : 2 LA	^{a}17.1	^{b}21.5	^{b}20.7	^{a}18.8	^{b}20.2
18 : 3 ALA	57.2	^{a}49.6	^{a}50.3	^{a}52.1	^{a}50.4
20 : 0	0.5	0.5	0.6	0.4	0.6
22 : 0	0.9	1.0	1.1	0.7	0.8
24 : 0	0.5	0.7	0.8	0.6	0.6

Note: FA – fatty acids, PA – palmitic acid, OA – oleic acid, LA – linoleic acid, ALA – α-linolenic acid
A one-way ANOVA statistical package was applied to evaluate the significance of differences between the FA contents. Significant differences were considered on the level $P < 0.05$. The differences between means assigned with the same letter are not statistically significant.
Poznámka: FA – mastné kyseliny, PA – kyselina palmitová, OA – kyselina olejová, LA – kyselina linolová, ALA – α-kyselina linolénová
Na vyhodnotenie významu rozdielom medzi obsahmi mastných kyselín sa použil štatistický balíček ANOVA. Významné rozdiely sa vzali do úvahy na úrovni $P < 0,05$. Rozdiely medzi priemermi v rámci toho istého písmena nie sú štatisticky významné.

Tabuľka 4 Zmeny zloženia mastných kyselín vo vzorkách rastlín počas pasienkovej sezóny v Trenčianskej Teplej v roku 2008
(1) dátum odberu, (2) mastné kyseliny

(19.0 °C) than in June 2007 (18.6 °C) as well as a lower rainfall in June 2008 (2.6 mm) than in June 2007 (3.5 mm). The higher milk CLA content in the end-May up to begin-July 2007 compared to 2008 in average of 1.8% and 1.4%, respectively, probably was associated with restricting pasture forage intake which can influence the ruminal biohydrogenation of consumed FA. The restricting forage intake would increase the mobilization of body fat in order to meet the ewes' energy demands. Mobilized body fat would increase the supply of CLA and TVA to the mammary gland and therefore increase the CLA milk content (Jiang et al., 1996).

Higher temperatures and lower rainfall affect pasture vegetation and thereby milk quality due to the pasture changes in content of FA precursors, biohydrogenated activity of rumen bacteria and rumen pH (Chilliard et al., 2002) as well as due to poorer availability of vegetation and correspondingly relative increase of concentrate added by milking to the ratio of total forage intake of ewes. Because of these factors, the ALA content in pasture in the meteorological more favourable pasture season 2008 did not change expressively as that in 2007. The effects of summer pasture period on ewes' milk FA profile in Ružomberok region were not significant because of superior meteorological conditions associated with higher altitude of pastures (800 m vs. 250 m a.s.l. in Trenčianska Teplá) (Ostrovský et al., 2009); the consistent milk content of CLA 2.5% was observed during whole summer pasture season (May – August 2007).

The LA/ALA ratio in pasture in Trenčianska Teplá during the pasture season 2007 increased from 0.23 in May to 0.42 (P < 0.001) in June, increased to 0.51 (P < 0.05) in August and decreased to 0.28 in September (similar ratio to that in May). Dhiman et al. (2000) stated that the CLA content increased in cows' milk when LA/ALA content ratio in feed was ≤ 1 : 1. Tyagi et al. (2007) reported similar data for buffalo milk. These results confirm that observed changes in the CLA content in ewes' milk throughout the pasture season are primarily determined by the ALA content in pasture.

Table 5 Changes in FA composition of ewes' milk during pasture season in the years 2007 and 2008

FA in g 100g^{-1} (2)	Sampling date (1)					
	2007			2008		
	May/9	July/30	Sept/13	May/12	June/30	Sept/17
PA	^{b}22.0	^{a}24.8	^{a}24.3	^{a}21.2	^{a}23.8	^{a}23.8
trans-10 18 : 1	0.22	^{a}0.14	^{a}0.13	^{b}0.37	^{c}0.31	^{c}b0.35
TVA	^{a}5.10	1.65	4.28	^{a}5.54	2.53	3.18
OA	^{a}18.1	^{b}22.7	^{c}20.1	^{a}18.4	^{b}23.3	^{c}19.0
LA	^{a}2.27	^{b}2.75	^{b}2.62	^{a}2.43	^{b}2.64	2.96
ALA	^{a}b1.13	1.57	^{a}1.25	^{b}1.07	1.33	1.78
CLA	^{a}2.33	^{b}1.33	^{a}2.27	^{a}2.27	^{b}1.31	2.03
Ratio						
TVA / CLA	2.19	1.24	1.89	2.44	1.94	1.57
TVA / *trans*-10 18 : 1	23.2	11.8	32.9	15.0	8.17	9.08

Note: FA – fatty acids, PA – palmitic acid, TVA – trans vaccenic acid, OA – oleic acid, LA – linoleic acid, ALA – α-linolenic acid, CLA – *cis*-9, *trans*-11 18 : 2
A one-way ANOVA statistical package was applied to evaluate the significance of differences between the FA contents. Significant differences were considered on the level P < 0.05. The differences between means assigned with the same letter are not statistically significant.

Poznámka: FA – mastné kyseliny, PA – kyselina palmitová, TVA – trans kyselina vakcénová, OA – kyselina olejová, LA – kyselina linolová, ALA – α-kyselina linolénová, CLA – *cis*-9, *trans*-11 18 : 2
Na vyhodnotenie významu rozdielom medzi obsahmi mastných kyselín sa použil štatistický balíček ANOVA. Významné rozdiely sa vzali do úvahy na úrovni P<0.05. Rozdiely medzi priemermi v rámci toho istého písmena nie sú štatisticky významné.

Tabuľka 5 Zmeny v zložení mastných kyselín v ovčom mlieku počas pasienkovej sezóny v rokoch 2007 a 2008
(1) dátum odberu, (2) mastné kyseliny

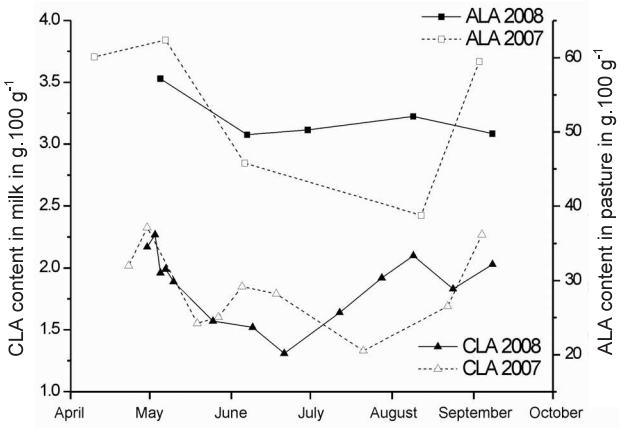

Figure 2 Changes in the content of *cis*-9, *trans*-11 18 : 2 (CLA) in ewes' milk and α-linolenic acid (ALA) content in pasture in g 100 g^{-1} during pasture season in 2007 and 2008

Obrázok 2 Zmeny v obsahu *cis*-9,*trans*-11 18 : 2 (CLA) v ovčom mlieku a v obsahu kyseliny α-linolénovej (ALA) v pasienku v g 100 g^{-1} počas pasienkovej sezóny v roku 2007 a 2008

In contrast to data published by Nudda et al. (2005) suggesting a decrease in the ALA content in ewes' milk during pasture season from 1.1% to 0.7%, we noted an increase in the ALA content in milk from 1.1% in May to 1.5% (P < 0.05) in August in the year 2007, and from 1.0% in May to 1.7% (P < 0.01) in August in the year 2008 (Figure 1). Different trends of changes in ALA content in ewes' milk during pasture season between Slovak and Italian pastures might have been caused by different rumen environment (bacteria type, pH) (Chilliard et al., 2002), which in our case decreased biohydrogenation activity in the rumen, thereby increasing the ALA content as the residual precursor in milk.

Changes in the composition of fatty acids in pasture after oversowing

By seeding grasses, legumes and their mixtures to pasture it is possible to increase both the quality of bulky food and the content of unsaturated FA precursors. Reseeding was realized in 2008 on the transition pasture in Trenčianska Teplá using common pasture oversowing. The changes in FA composition of pasture after reseeding the pasture mixture compared with the control are listed in Table 6. The ALA content in sowed forage changed less significantly compared with the control location. Pasture reseeding increased the ALA content in June by 3.8%, in July by 3.6%, in August by 0.5%, while that in September and October decreased by 4.6% and 5.5%, respectively. This relatively less significant change in the ALA content in pasture was related to the fact that ALA content in reseeding was similar to the original pasture. The composition of plant reseeding took into account the particular aspect of pasture production.

As the seasonal changes in CLA content of ewes' milk are determined primarily by the seasonal changes in ALA

Table 6 Changes in FA composition of pasture after reseeding the plants mixture compared with the control pasture in Trenčianska Teplá in 2008

Sampling date (1)	20/06/08		09/07/08		18/08/08		17/09/08		01/10/08	
FA in g 100 g^{-1} (2)	oversowing (3)	control (4)	oversowing (3)	control (4)	oversowing (3)	control (4)	oversowing (3)	control (4)	oversowing (3)	control (4)
10 : 0	–	–	–	–	0.1	–	–	–	0.2	–
12 : 0	0.2	0.2	0.1	0.1	0.3	0.4	0.3	0.2	0.6	0.4
14 : 0	0.6	0.6	0.3	0.4	0.6	1.3	1.2	1.3	1.2	1.3
15 : 0	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.3
16 : 0 PA	16.9	18.3	14.1	15.1	14.8	15.5	15.3	15.1	15.2	15.5
16 : 1	2.3	2.1	2.1	1.9	1.7	1.7	1.8	2.0	1.3	1.7
17 : 0	0.3	0.3	0.2	0.3	0.5	1.0	1.2	1.2	0.4	1.0
18 : 0	2.3	2.4	1.7	1.8	2.2	2.8	2.8	2.8	2.1	2.8
9*t* 18 : 1	0.4	0.4	0.2	0.4	0.5	0.4	0.5	0.5	0.2	0.4
9*c* 18 : 1 OA	3.4	3.9	2.2	2.2	2.7	3.8	4.2	3.8	4.5	3.8
18 : 2 LA	20.7	22.5	18.5	20.5	21.4	18.6	21.4	17.7	23.9	18.6
18 : 3 ALA	49.6	45.8	58.3	54.7	52.7	52.2	48.6	53.2	46.7	52.2
20 : 0	0.6	0.7	0.4	0.5	0.5	0.3	0.5	0.4	0.7	0.3
22 : 0	0.9	1.1	0.6	0.7	0.7	0.6	0.7	0.6	0.9	0.6
24 : 0	0.9	0.9	0.6	0.8	0.7	0.6	0.7	0.5	0.8	0.6

Note: FA – fatty acids, PA – palmitic acid, OA – oleic acid, LA – linoleic acid, ALA – α-linolenic acid
Poznámka: FA – mastné kyseliny, PA – kyselina palmitová, OA – kyselina olejová, LA – kyselina linolová, ALA – α-kyselina linolénová

Tabuľka 6 Zmeny v zložení mastných kyselín po príseve v porovnaní s kontrolným pasienkom v Trenčianskej Teplej v roku 2008
(1) dátum odberu, (2) mastné kyseliny, (3) s prísevkom, (4) kontrola

content in pasture plants, it is evident that modification of the plant composition in pastures by reseeding the plants with high ALA contents allows to increase the ALA content in pasture and consequently CLA, TVA and ALA contents in milk. Tables 2 and 3 show that *Trifolium repens* persist in the forage during the whole pasture season (from April to September) yet in different amounts (from 6% to 25% of the total number of plants) and the ALA content varies as well (from 48% to 63%). The fat content in *Trifolium repens* during pasture season is similar to that in pasture (Table 2). These results demonstrate the suitability of reseeding *Trifolium repens* in order to obtain more favourable healthy fatty acid composition in milk.

Elgersma et al. (2003) studied the changes in FA content in fresh and silaged *Lolium perenne*, which showed a very high content of ALA (69.4%) in the fresh state. In a previous study (Meľuchová et al. 2008), we found only a low content of this plant during the pasture season in Trenčianska Teplá (1% to 9%). In an extension of this study in 2008, the highest portion was found for *Lolium perenne* being a part of pasture mixture reseeding in experimental locality (10.3–11%) and *Trifolium repens* (7–9.4%) from grasses and legumes studied, respectively. *Lolium perenne* showed the quick spread in pasture (8% in the first year). Owing to quick connection to pasture, high cover, durability growth as well as high ALA content, *Lolium perenne* and *Trifolium repens* are suitable as reseeding plant species to improve the pasture quality in order to achieve a higher content of beneficial health-affecting FA, especially CLA, in ewes' milk. Optimization of oversowing the pasture with *Lolium perenne* and *Trifolium repens* in order to increase the ALA content, can reduce the impact of meteorological conditions on the ALA content in pasture, and thus stabilize the CLA content in ewes' milk during the summer pasture season in Trenčianska Teplá will be investigated in the next phase.

Conclusions

The FA content of natural grasslands, as well as floristical species changed significantly during the ewes' pasture season from April to September in two meteorological different years 2007 and 2008. The changes in CLA content in milk are determined primarily by the ALA content in pasture. The content of ALA being the most abundant FA in pasture decreased from May to summer and then increased until mid-September of 2007, and the CLA content changed correspondingly. The ALA pasture content changes in rainier and colder year 2008 during pasture season were significantly smaller but the CLA milk content was changed similarly than that in 2007. In terms of FA composition of ewes' milk fat including CLA content, the ewes' milk and related products (bryndza) made in September have a similar quality to that in May.

Súhrn

Zmeny v zložení mastných kyselín rastlinných druhov počas pasienkovej sezóny v dvoch meteorologicky rozdielnych rokoch a zmeny mastných kyselín v ovčom mlieku sa analyzovali plynovou chromatografiou. Zmeny obsahu *cis*-9,*trans*-11 18 : 2 (CLA) v mlieku sú primárne určované obsahom kys. α-linolénovej (ALA) v pasienkovom poraste. Obsah ALA ako najobsažnejšej mastnej kyseliny v pasienkovom poraste v suchšom roku 2007 klesal z polovice mája do polovice augusta zo 62,4% na 38,8% (g 100 g^{-1} rastlinného tuku) ($P < 0,001$) a v polovici septembra sa zvýšil na obsah ako v máji. Podobne, obsah CLA v mlieku z 2,3% (g 100 g^{-1}mliečneho tuku) v máji klesol na 1,3% ($P < 0,001$) v júli a následne sa zvýšil na 2,3% v septembri. V roku 2008, ktorý bol počas pasienkovej sezóny chladnejší a vlhší, najvyšší obsah CLA sa zistil tiež v máji (2,3%) a v sep-

tembri (2,0%), avšak minimálny obsah CLA v mlieku (1,3%) sa zistil v júni v zhode s vyššou priemernou teplotou a menšími zrážkami v porovnaní s júlom 2008. Podľa zloženia mastných kyselín ovčieho mlieka, najmä vzhľadom na obsah CLA, septembrové ovčie mlieko bolo podobnej kvality ako májové mlieko.

Kľúčové slová: zloženie mastných kyselín, pasienkové rastliny, ovčie mlieko, sezónne zmeny

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