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# QUALITY IMPROVEMENT OF SLOVAK EWE MILK BASED ON THE CONTENT OF HEALTH AFFECTING FATTY ACID COMPOUNDS

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## ABSTRACT

The content of 70 C4-C24 fatty acids (FA) in bulk milk samples of dairy ewes at 4 farms during pasture season and at one farm during winter season was determined by gas chromatography. The FA content in milk fat of grazed ewes was higher up to 4-fold for rumenic acid (CLA), 3-fold for trans-vaccenic acid (TVA) and 2-fold for α-linolenic acid (ALA) compared to that during winter diet. The content of CLA (3.5 %) and TVA (7.9 %) in milk fat of grazed ewes from Tajov farm was higher than that in milk of pasture grazed ewes or cows published previously. Nevertheless, the farm at Trenčianska Teplá because of lower altitude and corresponding climatic conditions in summer showed temporal variation up to 50 % lower content of CLA and TVA. The variations in CLA content during pasture season are primarily related to the seasonal changes of ALA content in pasture plants. These findings suggest that further development of ewes´ milk production should be situated in the northern part of Slovakia at higher altitudes thus providing better quality pastures and more consistent FA composition of ewe milk products with a high content of health affecting FA compounds. Ewe individuality is another important factor significantly affecting the FA milk fat content and also milk yield whereas the ewes´ breed and parity had only little effect. The effect of individuality, breeds and parity of grazed ewes based on FA content was investigated in herd of 148 Tsigai, 124 Improved Valachian and 56 Lacaune ewes grazing pasture and milk samples of individual ewes were taken on the same day. The CLA milk fat content varied up to 5-fold and milk yield up to 12-fold among individual ewes. An inverse relation between the milk yield and CLA content was observed which was not significant though. The ewes with a higher CLA milk fat content and a corresponding higher milk yield were considered in ewe selection for improving milk quality based on the content of health affecting FA compounds. Upon eliminating the data for 25 % ewes with a lower CLA milk fat content and lower milk yield the average CLA milk fat content increased by 10 % while keeping the milk yield.

**Key words:** ewes´ milk; fatty acids; CLA content; effect of diet; effect of individual ewes

## INTRODUCTION

The fatty acid (FA) profile of raw milk influences the nutritional and health characteristics of milk products. Milk FA have various effects on human health. The quality of dietary lipids could be an important modulator of the morbidity and mortality associated with obesity, arteriosclerosis, diabetes, hypertension, hyperlipidemia and cancer (Nagao and Yanagita, 2005). Conjugated FA have attracted considerable attention because of their potentially beneficial biological effects of attenuating these diseases. An increasing interest in enhancing the conjugated linoleic acid (CLA) content in food products arises from its potential anti-carcinogenic, anti-atherogenic, anti-diabetic, anti-obesity, and immunomodulatory functions observed in animal models. *Cis-9,trans-11* 18:2 is the major CLA isomer which represents 75 to 90 % of the total CLA in milk fat. This CLA isomer is an intermediate in the biohydrogenation of linoleic acid (LA), the main source

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in milk fat is from endogenous synthesis in the mammary gland. An intermediate in the rumen biohydrogenation of linoleic and linolenic acid (ALA) is *trans-vaccenic* acid, *trans*-1118:1 (TVA), which is converted to *cis*-9,*trans*-11 18:2 by enzyme Δ9-desaturase in the mammary gland. Polyunsatured fatty acids (PUFA), such as LA, 18:2, n-6, ALA, 18:3, n-3 and arachidonic acid (AA, 20:4, n-6) being essential FA are very important for maintaining biological functions in mammalians. The intake of n-3 highly unsaturated FA, such as eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), correlates with a reduced risk of cancer and cardiovascular disease. On the other hand, some saturated FA, such as myristic acid (MA, 14:0) and palmitic acid (PA, 16:0) and some unsaturated *trans*-FA show adverse effects on health maintenance and disease prevention.

There are several endogenous and exogenous factors affecting the ewes' milk FA profile, such as diet (Addis *et al*., 2005; Cabiddu *et al*., 2005), season (Nudda *et al*., 2005; Meľuchová *et al*., 2008), climate (Meľuchová *et al*., 2009), and physiological factors such as individuality of animals (Tsiplakou *et al*., 2006; Soják *et al*., 2012), breed (Signorelli *et al*., 2008; Soják *et al*., 2012), lactation stage and parity (Tsiplakou *et al.*, 2006; Soják *et al.*, 2012). Previous studies were focused mainly on most significant dietary sources of FA variation, whereas the effect of physiological and genetic factors, particularly the effect of individuality of ewes on milk FA profile was investigated to a lesser degree. Previous studies suggested that not only diet but also selection of individual ewes may serve as an important tool to improve the nutritional health quality of milk fat. In the light of recommendation aimed at lower intact of milk products (German and Dillard, 2004), contemporary research is focused on producing the milk with higher FA content that may have beneficial effects on human health. Carta *et al.* (2008) suggested that for the FA profile of ewes´ milk both classical quantitative genetic approach and genomic information based selection approaches will be realistic options in the future.

The most important product of ewe farming in Slovakia is a bryndza cheese with yearly production of about 5 x $10^6$ kg. The EC regulation No. 676/2008 of July 16, 2008 registered the name Slovenská bryndza in the Register of protected designations of origin and protected geographical indications. Nevertheless, the detailed composition of individual FA of bryndza cheese has not been established. The FA profile of bryndza cheese corresponds to those in the milk fat the product is being produced from. Therefore, the content of individual 70 C4-C24 FA in milk fat of ewes fed with total mixed ration (TMR) during winter as well as that of ewes grazing on natural pasture (experimental flock of 350 ewes) was measured as bulk milk samples. Effect of individuality of ewes as well as breed, parity and milk yield on the milk FA profile was investigated on the similar experimental flock belonging to three breeds-Tsigai, Improved Valachian, and Lacaune. In this case, the milk of grazed individual ewes was sampled on the same day due to the effects of differences in pasture management, botanical composition of pasture and vegetative stages of plants. The aim of this study was to determine the quality of Slovak ewe milk based on individual FA composition and to investigate options for improvement of ewe milk FA profile of those grazing on natural pasture as well as fed with winter diet.

## MATERIAL AND METHODS

Milk samples collected from ewes bred at Trenčianska Teplá farm were analyzed. This experimental farm kept 350 dairy ewes belonging to three breeds Tsigai, Improved Valachian, and Lacaune with parity 1-8. In winter season from mid-February to mid-April, the ewe basic diet consisted of corn silage (2.5 kg), meadow and Lucerne hay (0.7 kg and 0.3 kg), commercial concentrate feed mixture (0.8 kg), and mineral supplement (0.02 kg). In the grazing season from mid-April to mid-September, the ewes grazed natural pasture and also received a concentrated feed at a dose of 0.2 kg/day during machine milking. The FA composition of bulk ewe milk from the daily milkings was analyzed at least once in a month, and more frequently in milk of ewes fed TMR and at the beginning of the pasture season (April-June during 2007-2009). On days of milk sampling during pasture season, botanical families and main plant species of pasture samples on the content of individual FA were also analyzed (Meľuchová *et al*., 2008). For evaluation of the effect of ewe individuality on FA profile, individual milk samples from 328 ewes, 148 Tsigai, 124 Improved Valachian, and 56 Lacaune, grazing pasture were collected on the same day on morning milking in the year 2009. For completion of data evaluation, the bulk milk products of ewes grazed on pasture from Ružomberok, Liptovská Anna, and Tajov farms breeding 300-800 ewes were analyzed, too.

The lipids from milk samples and dried pasture plant samples were extracted using chloroform-methanol mixture (2:1). The extracts were derivatized by sodium methanolate in methanol and analyzed as methyl esters of fatty acids (FAME). For analysis of FAME from bulk milk samples capillary gas chromatography (GC) with flame ionization detector in capillary column 100 m x 0.25 mm i.d. x 0.25 μm film thickness of CP-Sil 88 (Varian, Palo Alto, CA, USA) as stationary phase was used. Gas chromatographically unseparated CLA isomers, mainly triplet *trans*-7,*cis*-9 / *cis*-9,*trans*-11 / *trans*-8,*cis*-10 isomers of CLA, were resoluted by chemometric deconvolution (Blaško *et al*., 2009). For quick analysis of milk FA of individual ewes the capillary column of 60 m x 0.25 mm i.d. x 0.25 µm film thickness of DB-23 (J&W Scientific, Agilent Technologies, USA) as stationary phase was used. Individual FAME separation was performed on a gas chromatograph Agilent Technologies 6980N (Agilent, Waldbrown, Germany) and a 5973 Network mass-selective detector and at a programmed column temperature of 70-240°C. Separated FAMEs were identified by reference materials, published retention data and mass spectrometric measurements. The chromatograms were evaluated quantitatively using a method of internal normalization and published response factors of flame ionization detector for FAME (Ackman, 2002). The FAME composition of milk fat was expressed as grams of each individual FAME per 100 g of sum detected FAME. The average relative standard deviation of analyzed FAME with content > 0.5 g 100 $g^{-1}$ was 1.1 % for whole analytical procedure and 5 replicate milk samples.

The contents of individual FA samples in bulk milk were analyzed using a one-way ANOVA statistical package. The data for individual animals were statistically evaluated using a general linear model procedure with nominal variables of breed and parity and continuous variable of milk yield. Significant differences were considered at the level $P < 0.05$.

## RESULTS AND DISCUSSION

The results of GC analysis of individual FA content in bulk milk samples of ewes fed with winter diet (TMR) and those grazed on natural pasture as well as that of pasture plant samples are presented in Table 1. The variations in CLA milk fat content during continuous transition from a period of TMR diet, a period of gradually increased content ratio of pasture /TMR, and a final period of pasture feeding are presented in Fig. 1.

*Cis*-9,*trans*-11 CLA isomer and *trans*-vaccenic acid (TVA) showed more than 5-fold changes in the individual FA milk fat content among ewes fed winter diet and pasture which was the most significant. Ewe feeding with TMR during the first 60 days after lambing, except for colostrum period, resulted almost in consistent individual milk FA content, e.g., CLA 0.65 g 100 $g^{-1}$ in agreement with a quasi-standardized TMR diet. Nevertheless, the ALA content of meadow hay as a part of TMR diet from various cuts of 32 Slovak producers was in broad content range from 14.6 to 53.5 % (g 100 $g^{-1}$ FAME). Replacement of meadow hay as a part of TMR diet by that having a higher ALA content (50 g 100 $g^{-1}$ versus 25 g 100 $g^{-1}$) led to a two-fold increase of CLA (0.65 to 1.22 g 100 $g^{-1}$ FAME) and TVA (1.44 to 2.58 g 100 $g^{-1}$ FAME) content in milk of TMR-fed ewes. Luna *et al.* (2008) reported a two-fold increase in CLA and TVA contents upon feeding ewes with a TMR diet enriched with flax seed and sunflower oil (CLA 0.47 vs. 0.85 g 100 $g^{-1}$, and TVA 0.87 vs. 1.72 g 100 $g^{-1}$ FAME). Generally, when compared with previously published data, the estimated CLA milk fat content in our ewes fed with a winter diet (0.65 g 100 $g^{-1}$ FAME) was moderately higher due to more appropriate TMR composition.

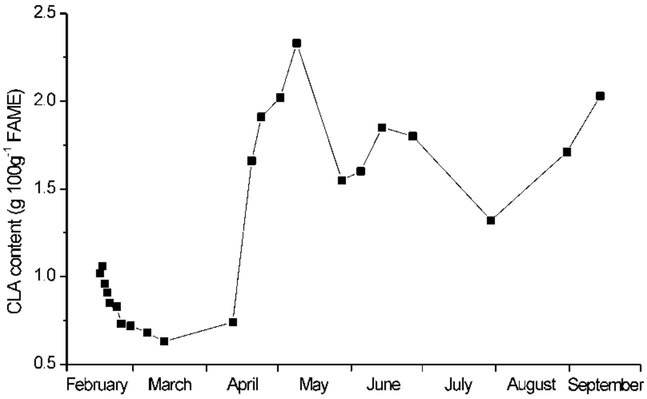


**Fig. 1: Temporal variations in CLA milk fat content of ewes during a period of TMR diet, a period of gradually increased content ratio of pasture / TMR diet, and a period of pasture feeding in Trenčianska Teplá farm.**

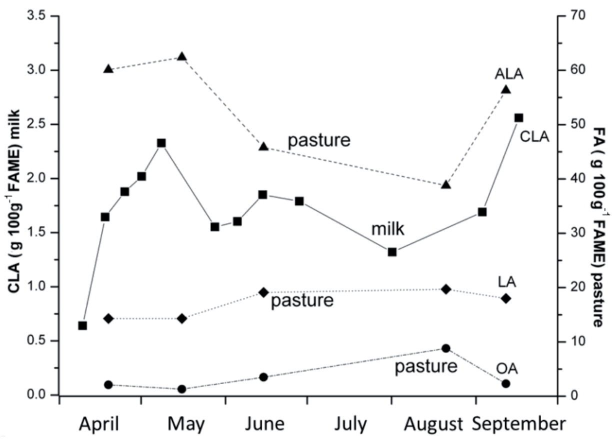


**Fig. 2: Temporal variations in the content of CLA in ewe milk fat and content of α-linolenic (ALA), linoleic (LA) and oleic (OA) acids in pasture samples during pasture season in Trenčianska Teplá.**

**Table 1: Milk fatty acid composition of ewes fed TMR, hay TMR aditived, grazing on pasture in TrenčianskaTeplá, Ružomberok, Tajov, Liptovská Anna and in pasture plants (g 100 g$^{-1}$ FAME)**

| | | | Trenč. Teplá | | Ružomberok | Tajov | Lipt. Anna | Pasture (T. Teplá) |
|---|---|---|---|---|---|---|---|---|
| | TMR | TMR+hay | May | July | July | May | September | May |
| C4:0 | 3.31 | 2.58 | 4.05 | 2.25 | 2.83 | 2.42 | 2.52 | - |
| C6:0 | 2.43 | 2.58 | 2.84 | 1.29 | 1.75 | 1.91 | 1.75 | - |
| C9:0 | 0.04 | 2.69 | 0.04 | 0.04 | 1.46 | 1.78 | 1.71 | - |
| C8:0 | 2.23 | 0.08 | 2.78 | 1.01 | 0.04 | 0.04 | 0.05 | - |
| C10:0 | 6.82 | 8.50 | 8.45 | 3.32 | 4.53 | 5.93 | 6.18 | 0.04 |
| C10:1 | 0.16 | 0.02 | 0.25 | 0.15 | 0.01 | 0.01 | 0.02 | - |
| C11:0 | 0.08 | 0.10 | 0.11 | 0.03 | 0.05 | 0.06 | 0.08 | - |
| C12:0 | 4.01 | 4.41 | 4.80 | 2.38 | 2.67 | 3.27 | 4.09 | 0.12 |
| C12:1 | 0.02 | 0.04 | 0.03 | 0.09 | 0.02 | 0.03 | 0.01 | - |
| iso-C13:0 | 0.06 | 0.10 | 0.07 | 0.05 | 0.07 | 0.09 | 0.05 | - |
| C13:0 | 0.09 | 0.09 | 0.11 | 0.08 | 0.08 | 0.07 | 0.09 | - |
| iso-C14:0 | 0.11 | 0.12 | 0.12 | 0.13 | 0.14 | 0.15 | 0.10 | - |
| C14:0 (MA) | 9.21 | 8.84 | 9.92 | 9.72 | 8.50 | 9.61 | 11.12 | 0.42 |
| C14:1 | 0.10 | 0.17 | 0.16 | 0.20 | 0.13 | 0.16 | 0.31 | 0.01 |
| iso-C15:0 | 0.27 | 0.34 | 0.39 | 0.43 | 0.36 | 0.36 | 0.36 | - |
| anteiso-C15:0 | 0.37 | 0.36 | 0.67 | 0.57 | 0.65 | 0.70 | 0.50 | - |
| C15:0 | 0.83 | 0.76 | 1.14 | 1.23 | 1.18 | 1.18 | 1.13 | 0.11 |
| C15:1 | 0.05 | 0.03 | 0.12 | 0.14 | 0.15 | 0.13 | 0.01 | - |
| iso-C16:0 | 0.27 | 0.24 | 0.29 | 0.27 | 0.31 | 0.30 | 0.23 | - |
| C16:0 (PA) | 22.85 | 21.65 | 18.05 | 24.82 | 22.15 | 21.73 | 22.14 | 15.16 |
| 6-8t-C16:1 | 0.08 | 0.18 | 0.61 | 0.27 | 0.53 | 0.69 | 0.48 | - |
| 9-11t-C16:1 | 0.34 | 0.42 | 0.39 | 0.40 | 0.55 | 0.40 | 0.50 | - |
| c9-C16:1 | 0.71 | 1.19 | 0.72 | 1.11 | 0.74 | 0.79 | 1.04 | 2.01 |
| c11-C16:1 | 0.05 | 0.06 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 |
| iso-C17:0 | 0.52 | 0.54 | 0.61 | 0.60 | 0.55 | 0.56 | 0.51 | - |
| anteiso-C17:0 | 0.47 | 0.49 | 0.55 | 0.48 | 0.45 | 0.49 | 0.34 | - |
| C17:0 | 0.66 | 0.70 | 0.76 | 0.83 | 0.82 | 0.78 | 0.63 | 0.17 |
| C17:1 | 0.26 | 0.46 | 0.22 | 0.38 | 0.23 | 0.26 | 0.25 | 0.05 |
| iso-C18:0 | 0.09 | 0.12 | 0.04 | 0.07 | 0.06 | 0.06 | 0.04 | - |
| C18:0 | 12.01 | 6.26 | 9.66 | 12.30 | 11.52 | 10.54 | 9.22 | 1.58 |
| t6-t8-C18:1 | 0.10 | 0.18 | 0.08 | 0.07 | 0.18 | 0.19 | 0.16 | - |
| t9-C8:1 | 0.14 | 0.26 | 0.12 | 0.10 | 0.21 | 0.24 | 0.18 | - |
| t10-C18:1 | 0.35 | 0.63 | 0.21 | 0.14 | 0.36 | 0.18 | 0.33 | 0.15 |
| t11 C18:1 (TVA) | 1.44 | 2.58 | 4.68 | 1.70 | 5.87 | 7.85 | 5.25 | - |
| t12/c6-8-C18:1 | 0.24 | 0.31 | 0.21 | 0.09 | 0.44 | 0.29 | 0.39 | - |
| c9/c10-C18:1 (OA) | 21.43 | 22.95 | 17.16 | 24.19 | 17.53 | 15.52 | 15.66 | 2.55 |
| c11/t15-C18:1 | 0.66 | 0.65 | 0.51 | 0.57 | 0.70 | 0.55 | 0.62 | 0.29 |
| c12-C18:1 | 0.36 | 0.36 | 0.27 | 0.26 | 0.26 | 0.16 | 0.31 | - |
| c13-C18:1 | 0.09 | 0.12 | 0.13 | 0.08 | 0.15 | 0.14 | 0.12 | - |
| c14-C18:1+9t12t-C18:2 | 0.36 | 0.28 | 0.61 | 0.52 | 0.81 | 0.63 | 0.76 | - |
| C18:2 | 0.35 | 0.36 | 0.66 | 0.55 | 0.59 | 0.41 | 0.75 | - |
| c9t12-C18:2 | 0.11 | 0.17 | 0.16 | 0.16 | 0.20 | 0.13 | 0.21 | - |
| t9tc12 + t11c15-C18:2 | 0.08 | 0.12 | 0.13 | 0.08 | 0.08 | 0.09 | 0.12 | - |
| C18:2 n-6 (LA) | 2.77 | 3.15 | 2.43 | 2.75 | 3.05 | 2.12 | 2.79 | 16.48 |
| t11c15-C18:2 | 0.14 | 0.09 | 0.11 | 0.11 | 0.10 | 0.07 | 0.06 | - |
| c9c15-C18:2 | 0.04 | 0.04 | 0.09 | 0.08 | 0.13 | 0.09 | 0.11 | - |
| C18:3 n-6 | 0.02 | 0.01 | 0.03 | 0.02 | 0.01 | 0.02 | 0.02 | - |
| C19:0+C18:2 | 0.13 | 0.10 | 0.12 | 0.15 | 0.05 | 0.03 | 0.03 | - |
| C18:3+cyclo C18 | 0.14 | 0.11 | 0.22 | 0.29 | 0.11 | 0.11 | 0.09 | - |
| C18:3 n-3 (ALA) | 0.52 | 0.56 | 1.08 | 1.57 | 1.72 | 1.01 | 1.88 | 58.96 |
| t7c9-C18:2 | 0.020 | 0.04 | 0.03 | 0.04 | 0.04 | 0.06 | 0.04 | - |

MA – myristic acid; PA – palmitic acid; TVA – *trans*-vaccenic acid; OA – oleic acid; LA – linoleic acid; ALA – α-linolenic acid

**Table 1: (continuous)**

| | TMR | TMR+hay | Trenč. Teplá May | Trenč. Teplá July | Ružomberok July | Tajov May | Lipt. Anna September | Pasture (T. Teplá) May |
|---|---|---|---|---|---|---|---|---|
| c9t11-C18:2 (CLA) | 0.65 | 1.22 | 2.49 | 1.21 | 2.62 | 3.50 | 2.53 | - |
| t8c10-C18:2 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | - |
| t9c11-C18:2 | 0.005 | 0.011 | 0.006 | 0.006 | 0.011 | 0.001 | 0.011 | - |
| t10c12-C18:2 | 0.001 | 0.002 | 0.002 | 0.001 | 0.003 | 0.008 | 0.003 | - |
| t11c13-C18:2 | 0.006 | 0.003 | 0.04 | 0.020 | 0.07 | 0.12 | 0.07 | - |
| c9c11-C18:2 | 0.005 | 0.006 | 0.003 | 0.003 | 0.006 | 0.005 | 0.006 | - |
| t12t14-C18:2 | 0.002 | 0.001 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | - |
| t11t13-C18:2 | 0.006 | 0.01 | 0.02 | 0.0 | 0.05 | 0.02 | 0.04 | - |
| t7t9-t10t12-C18:2 | 0.03 | 0.002 | 0.02 | 0.012 | 0.02 | 0.01 | 0.02 | - |
| C20:0 | 0.22 | 0.13 | 0.21 | 0.32 | 0.28 | 0.25 | 0.15 | 0.47 |
| C20:1 | 0.10 | 0.09 | 0.20 | 0.12 | 0.20 | 0.23 | 0.03 | - |
| C20:2 | 0.03 | 0.03 | 0.02 | 0.03 | 0.02 | 0.01 | 0.02 | - |
| C21:0 | 0.06 | 0.04 | 0.11 | 0.10 | 0.12 | 0.10 | 0.09 | - |
| C20:3 n-6 | 0.03 | 0.06 | 0.08 | 0.03 | 0.03 | 0.02 | 0.02 | - |
| C20:4 n-6 (AA) | 0.24 | 0.33 | 0.21 | 0.20 | 0.15 | 0.08 | 0.09 | - |
| C22:0 | 0.07 | 0.03 | 0.14 | 0.14 | 0.18 | 0.18 | 0.11 | 0.83 |
| C20:5 n-3 (EPA) | 0.05 | 0.06 | 0.07 | 0.10 | 0.09 | 0.08 | 0.09 | - |
| C23:0 | 0.05 | 0.01 | 0.10 | 0.09 | 0.12 | 0.14 | 0.08 | - |
| C24:0 | 0.05 | 0.01 | 0.17 | 0.06 | 0.11 | 0.13 | 0.06 | 0.57 |
| C22:5 n-3 (DPA) | 0.01 | 0.15 | 0.21 | 0.17 | 0.19 | 0.15 | 0.19 | - |
| C22:6 n-3 (DHA | 0.02 | 0.04 | 0.07 | 0.04 | 0.05 | 0.05 | 0.08 | - |

CLA – conjugated linoleic acid; AA – arachidonic acid; EPA – eicosapentaenoic acid; DPA – docosapentaenoic acid; DHA – docosahexaenoic acid.

Table 1 and Fig. 1 suggest a rise in CLA, TVA and ALA content in milk samples upon transition from a TMR diet to a pasture diet. CLA, TVA and ALA values have been increasing from a TMR period throughout a transition period to the beginning of pasture season (May) ($P < 0.001$). The CLA and TVA contents declined later (June-July) ($P < 0.001$), however, they rose again to the end of the pasture season from August to mid-September ($P < 0.001$).The composition of FA in the milk of pasture-grazed ewes in September was similar to that at the beginning of pasture season in May. The content of CLA and TVA in milk of ewes grazing fresh pasture at the beginning or at the end of pasture season was up to 4-fold higher ($P < 0.001$) and that of ALA was double compared with those fed with standard TMR diet ($P < 0.001$). These findings were consistent with a higher ALA content in pasture (60 g 100 $g^{-1}$ FAME) (Fig. 2). Lower CLA (up to 2-fold) and TVA contents in milk fat were noted during warm and dry summer (June-July) which were associated with the corresponding decrease in ALA content (60 vs. 40 g 100 $g^{-1}$ FAME) in grazed pasture. About 2-fold increase in CLA and TVA contents ($P < 0.001$) in ewes' milk fat noted between the end of July and mid-September was consistent with higher ALA content of pasture grass (60 vs. 40 g 100 $g^{-1}$ FAME). Of note, Nudda *et al*. (2005) published a regular monotonic decrease of ewe milk CLA content during pasture season from 2.20 in March to 1.14 g 100 $g^{-1}$ FAME in June. The contents of CLA (3.50 %) and TVA (7.85 %) in milk fat of ewes grazed on Tajov pasture were higher than those of cows and ewes grazing on pasture published so far (Collomb *et al.*, 2006).

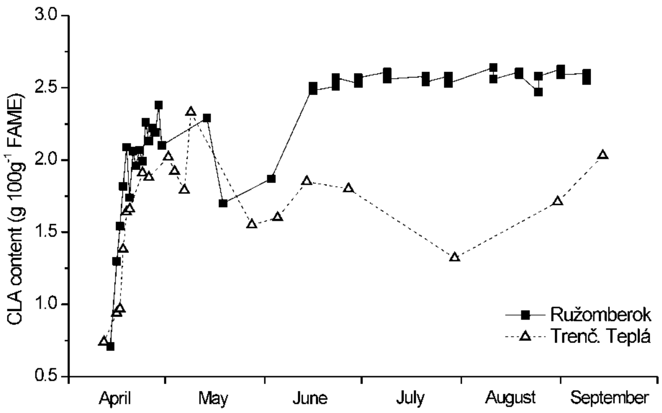


**Fig. 3: Temporal variations in the content of CLA ewe milk fat during pasture season in ewe farm Trenčianska Teplá (250 m a.s.l.) and Ružomberok farm (800 m a.s.l.).**

Similar data on milk FA composition of ewes grazing pasture at Trenčianska Teplá in May and September were also found in other ewe farms in Ružomberok, Liptovská Anna and Tajov (Table 1). The comparison of seasonal variations of CLA milk fat content in Ružomberok and Trenčianska Teplá farms shown in Fig. 3 suggest that the effect of summer pasture period on CLA content from Ružomberok farm was not significant because of superior climate conditions associated with higher altitude of pastures (altitude: 800 m vs. 250 m a.s.l.). Ewes grazing pastures at higher altitudes (altitude: 800 m a.s.l.) in North Slovakia showed a more consistent composition of milk FA in the same pasture season. Bearing in mind global warming, the above findings suggest that further development of ewes' milk production should be situated in a northern part of the country at higher altitudes thus providing more good-quality pasture and meadow hay for a dry winter diet as well as more consistent FA composition of ewe milk products with a higher content of health affecting FA compounds (Ostrovský *et al.*, 2009).

The observed CLA-ALA relationships suggest that the variation of CLA milk fat content during pasture

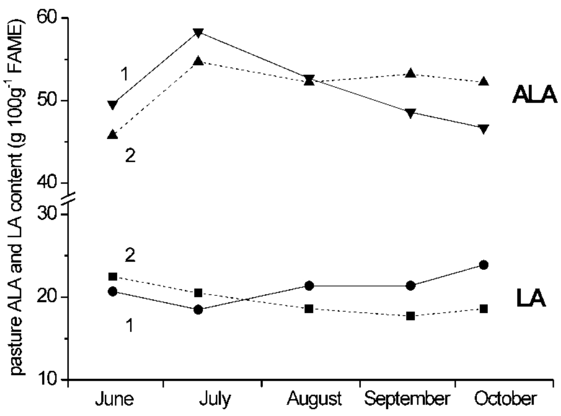


**Fig. 4: Temporal variations in the content of ALA and LA of pasture after reseeding the plants mixture compared with the control pasture in Trenčianska Teplá in 2008, 1 – oversowing pasture, 2- control pasture.**

| Month/year | Average daily temperature [°C] | | | Average daily rainfall [mm] | | | Average CLA content [g 100g$^{-1}$] | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2009 | 2007 | 2008 | 2009 | 2007 | 2008 | 2009 |
| April | 11.0 | 10.0 | 12.5 | 0.1 | 1.5 | 0.4 | 2.00 | 1.02 | 2.00 |
| May | 16.4 | 14.8 | 14.5 | 3.0 | 1.8 | 2.0 | 2.30 | 1.93 | 1.70 |
| June | 18.6 | 19.0 | 16.5 | 3.5 | 2.6 | 5.2 | 1.65 | 1.47 | 2.00 |
| July | 19.4 | 19.2 | 19.8 | 2.3 | 3.5 | 3.0 | 1.32 | 1.64 | 2.05 |
| August | 19.4 | 18.3 | 19.5 | 2.5 | 2.6 | 1.8 | 1.71 | 2.01 | 1.88 |

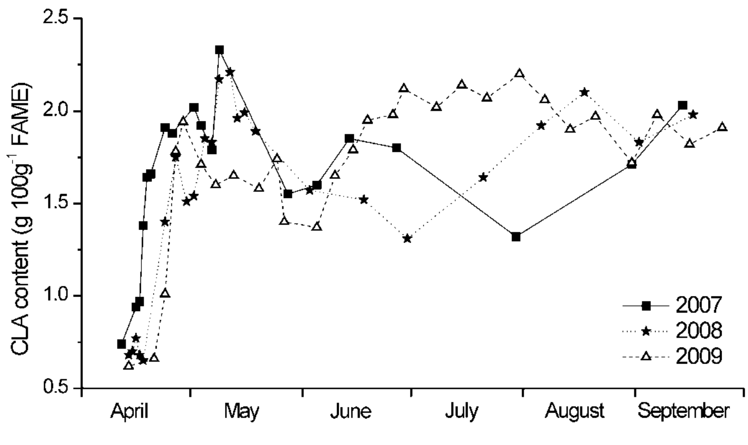


**Fig. 5: Temporal variations of average daily temperature, average daily rainfall, and average CLA milk fat content during pasture season of years 2007, 2008 and 2009 in Trenčianska Teplá farm.**

season are primarily related to seasonal changes of ALA content in pasture plants. Preliminary pasture reseeding in Trenčianska Teplá farm was accomplished using a common oversowing mixture of seven plant species (32 kgha$^{-1}$). Pasture reseeding resulted in a relative increase of 8 % in pasture ALA content in June-July, however, it decreased to a control level throughout pasture season (Meľuchová *et al.*, 2009) (Fig. 4). In the next season we will investigate optimized oversowing the pasture with *Lolium perenne* and *Trifolium repens* showing quick connection to pasture, high cover and durability growth aimed at increasing the ALA content in pasture.

The FA composition of ewe milk samples collected at 4 Slovak ewe farms (Trenčianska Teplá, Ružomberok, Liptovská Anna and Tajov) (altitude: 250 – 800 m a.s.l.) were compared with published data for cow milk collected at 4 Swiss alpine locations (altitude: 1275 – 2120 m a.s.l.) (Kraft *et al.*, 2003). The milk and cheese contents of CLA, TVA and ALA were found to be rather similar despite significant altitude and botanical differences between these pastures. Consequently, altitude or botanical composition does not serve as a crucial factor for increasing the content of health promoting milk FA. A similar content of ALA in Slovak and Swiss pastures (about 60 %) provides comparably high CLA, TVA, and ALA contents in milk products of ewes and cows grazing on pasture. *Trans*-11, *cis*-13 CLA was the second most abundant CLA isomer in milk fat from cows grazing high-altitude Alpine pastures. Therefore, this isomer has been implicated as a useful indicator of milk products of Alpine origin. Nevertheless, we noted identical data for milk of ewes grazing on natural pastures with an altitude range of 250-800 m a.s.l. (Blaško *et al.*, 2009; Soják *et al.*, 2009). The content of *trans*-11,*cis*-13 CLA isomer in milk fat of ewes grazing on pasture is about 3-fold higher than that of *trans*-7,*cis*-9 CLA isomer being normally the second-most abundant CLA isomer in ruminant milk fat.

Further improvement in FA milk fat profile can be achieved by selecting individuals from ewes' flock with higher CLA milk fat content and higher milk yield. The current selection of dairy ewes does not consider the content of individual FA in milk fat. The study on the effects of inter-individual variation, breed, parity on milk FA profile and milk yield of individual ewes grazed on pasture of 328 ewes belonging to Tsigai (148), Improved Valachian (124), and Lacaune (56) breeds shows how these parameters might affect the composition of individual milk fat FA (Soják *et al.*, 2012). To eliminate confounding dietary and seasonal effects on milk FA profile, the milk of ewes grazed on pasture was sampled on the same day (May 16, 2009). On the same day, the highest CLA milk fat content was found during 2007-2008 years (Fig. 5).

In addition to diet, ewe individuality is another most important factor significantly affecting the milk FA profile. The CLA milk fat content varied up to 5-fold among individual 328 ewes (from 0.5 to 2.5 g 100 g$^{-1}$) feeding pasture on the same day (Fig. 6).The quasi Gaussian frequency distribution of individual ewes for CLA milk fat content of the whole flock, and separately for Tsiagi, Improved Valachian, and Lacaune breeds of this flock was found. In addition, the systematic differences in CLA milk fat content between ewe pairs of examined breeds were prevailingly maintained over 16-wk pasture season as well as in previous year (2008) (Fig. 7). Earlier, Peterson *et al.* (2002) and Kelsey *et al.* (2003) stated that cows maintained a quite consistent hierarchy in CLA milk fat content over time when cows fed with the same diet even when cows were switched between the diets that give substantial differences in CLA milk fat content. A profound impact of ewe individuality inhibits a less significant effect of breed and parity on milk FA composition.

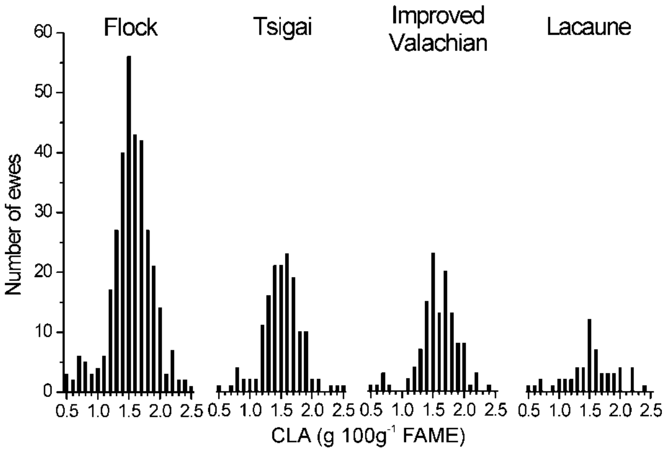


**Fig. 6: Frequency distribution for CLA milk fat content of 328 individual ewes including 148 Tsigai, 124 Improved Valachian, and 56 Lacaune breeds of this flock May 16, 2009 in Trenčianska Teplá farm.**

The study on effect of ewe breed on milk FA profile can be investigated for producing ewe cheese with different sensory properties (Signorelli *et al.*, 2008). In our study, the average content of CLA and other FA of the whole flock and that of Tsigai, Improved Valachian and Lacaune breeds was similar and prevailingly not statistically different. Similarly, in the study of the effect of breed on milk FA content in Awassi, Lacaune, Friesland and Chios breeds, Tsiplakou *et al.* (2008) found no effect of ewes' breed on milk FA profile.

Animal parity is another physiological factor, which can affect FA profile in milk fat. Our results in

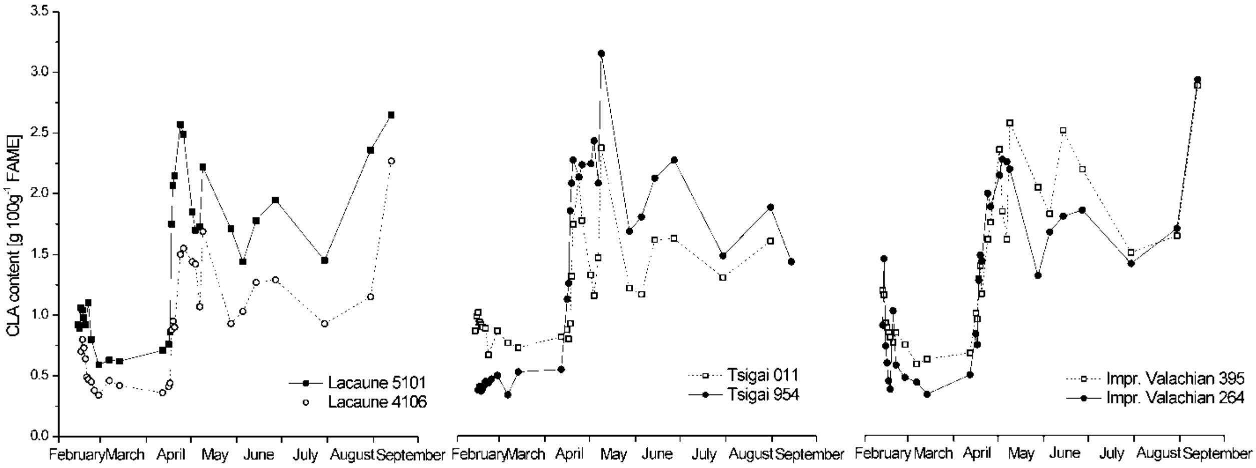


**Fig. 7: Temporal variations in CLA milk fat content of pairs Lacaune, Tsigai and Improved Valachian ewes with most different CLA milk fat contents (from 15 investigated ewes) during continuous transition from dry winter to natural pasture diet.**

ewes with parities 1-8 suggest a 60 % decrease in milk yield between ewes with 1-3 parities and those with 7 parities (Soják *et al.*, 2012). A higher content of oleic acid by 13 % and lower content of myristic acid by 8 % was the most significant difference between primiparous and multiparous ewes in the entire flock and also in individual breeds. The content of CLA slightly changed with parity: it increased with parity 1-3 and then decreased with parities 6-7. The data document that ewe parities 1-3 have a little impact on FA milk fat profile.

Ewe individuality markedly affected milk yield with variations up to 12-fold (100-1250 mL at the morning milking). Similarly, Haenlein (2001) found the differences in ewes´ milk yield about 10-fold. The average morning milk yield of investigated flock was 627 mL, decreased in the order of Lacaune (711 mL) < Improved Valachian (611 mL) ≈ Tsigai (609 mL). The quasi Gaussian frequency distribution of individual ewes as for CLA milk fat content of the whole flock, and separately for Tsigai, Improved Valachian and Lacaune breeds of this flock was observed also for milk yield (Fig. 8), as well as Δ9-desaturase index and atherogenicity index (Soják *et al.*, 2012). Just like in the year 2008, the rank of individual ewes during pasture season based on milk yield was to a large extent maintained particularly for those with more different milk yield.

The calculated linear regression equation between ewes' milk yield and CLA milk fat content in individual ewes of investigated breeds suggested a non-significant inverse relationship between the CLA milk fat content and milk yield (Fig. 9). There seems to be only a trend of decreasing CLA milk fat content with increasing milk yield. Therefore, the ewes with a higher CLA milk fat content and correspondingly higher milk yield should be considered in ewe selection. The annual replacement rate in an experimental ewe flock is 20-25 %. Upon eliminating the data for 25 % ewes with a lower CLA milk fat content and a lower milk yield, the average CLA milk fat content increased approximately by 10 % while keeping the

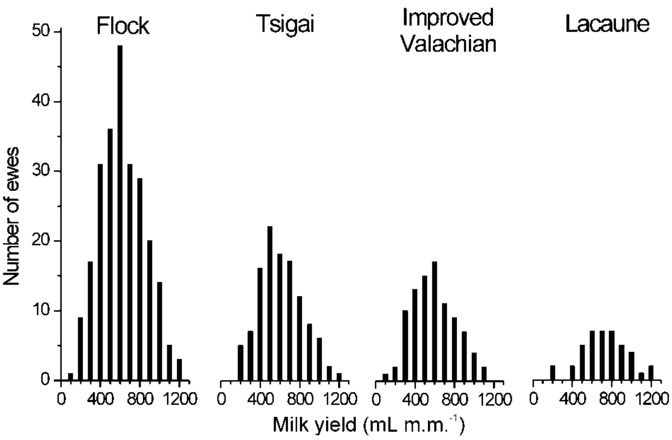


**Fig. 8: Frequency distribution for milk yield (mL morning milking) of 328 individual ewes of flock and 148 Tsigai, 124 Improved Valachian, and 56 Lacaune breeds on May 16, 2009 in Trenčianska Teplá farm.**

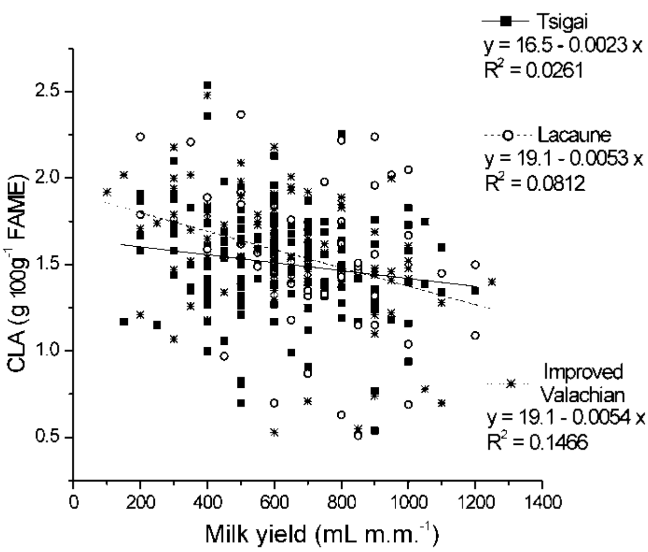


**Fig. 9: Relationship between CLA milk fat content and yield of morning milk of 328 individual ewes 148 Tsigai, 124 Improved Valachian, and 56 Lacaune breeds on May 16, 2009 in Trenčianska Teplá farm.**

milk yield. In the case of higher starting CLA milk fat content this increase should be even higher. On the other hand, the average milk yield increased approximately by 15 % while keeping the average CLA milk fat content.

## CONCLUSION

The contents of CLA and TVA in milk fat of pasture-grazed ewes belong to the highest ever published for ruminant animals. They decreased in lowland pastures during summer months, and increased again at the end of pasture season up to the levels noted at the beginning of the season. The seasonal variations in milk FA contents were related to those of ALA content in pasture. The strategies aimed at increasing CLA milk fat content in ewes fed with a winter diet through replacing the meadow hay as a part of a winter diet with that having a higher ALA content, and in those grazing on pasture through reseeding pasture with plant species with a higher ALA content were investigated. In addition to diet, ewe individuality is another important factor significantly affecting the CLA milk fat content. Ewe individuality significantly affected milk yield too. Upon consideration of statistically not significant relationship between the CLA milk fat content and milk yield, the animals with a higher CLA milk fat content and a correspondingly higher milk yield should be considered in ewe selection for improving milk quality in experimental ewe flock. The ewe selection based on a single milk sample for each ewe on the same day from the experimental grazed flock was substantiated by prevailing maintenance of the systematic differences in CLA milk fat content as well as milk yield of individual ewes during pasture season as well as between pasture seasons.

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