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The effect of parity on the proportion of important healthy fatty acids in raw milk of Holstein cows

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Summary

The objective of this study was to determine and evaluate the effect of parity on the fatty acids groups' proportion in Holstein cows' milk during the first phase of lactations, with an emphasis on its potential importance for consumer health. A total of 25 Holstein cows, 9 primiparous, 9 in the 2nd, and 7 in the 3rd and subsequent parity, were observed and sampled at 7-day intervals through the first 17 weeks of lactation. The percentage proportion of saturated (hypercholesterolemic and volatile as its components) and unsaturated (monounsaturated and polyunsaturated as its components) fatty acids in the samples of milk fat (n=425) was determined. The effects of parity and negative energy balance, as well as regression, on the lactation week and the fat to protein ratio were evaluated using SAS 9.3. A significantly (P<0.01) lower proportion of unhealthy hypercholesterolemic fatty acids was detected in primiparous cows (-2.67 %) and those in the 3rd and subsequent lactation (-2.94 %) compared to the 2nd lactation, as well as a simultaneously higher proportion of healthy unsaturated fatty acids (+2.07, respectively +3.08 %). The determined relationships corresponded to organism stress evoked by the initiation of milk production and its maintenance in higher parities. Therefore, the generally required prolongation of dairy cows' longevity can influence on the quality of raw milk, especially considering composition of fatty acids.

Key words: cattle, longevity, milk production, milk quality, milk fat composition

Introduction

Production of milk used for subsequent processing is a basic ability of ruminant females (Szencziová et al., 2013) and directly affects the economic efficiency of their breeding. The level of dam milk yield and milk composition, as well as the quality, are affected by different genetic (Stádník and Louda, 1999; Ivkić et al., 2012; Ivanova et al., 2013; Kadlecová et al., 2014), environmental (Klir et al., 2012), and animals' individual factors such as lactation number and/or phase (Zamberlin et al., 2012; Toušová et al., 2013), health (Vacek et al., 2007), metabolism intensity (Ducháček et al., 2012a, b; Ducháček et al., 2013), udder morphology (Stádník et al., 2010) or even temperament (Gergovska et al., 2012).

Among other traits, milk fat proportion is a basic criterion for raw cow's milk encashment and profitability (Bašić et al., 2012). Therefore, its total content is very important for breeders. Milk fat is represented by the content of individual fatty acids (FAs) and their triglycerides (Marounek et al., 2012). FAs divided into individual groups, saturated (SFAs) and unsaturated (UFAs), as a part of food for humans were studied and commented on, focusing their effect on consumers' health (Staňková et al., 2013). The negative effect of SFAs on the cardiovascular system is generally known. Mainly

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the hypercholesterolemic fatty acids (HCFAs) as a proportion of it cause fat deposition in the vascular walls, subsequently followed by a higher risk of atherosclerotic diseases (Jensen, 2002). UFAs, mono- (MUFAs), as well as polyunsaturated fatty acids (PUFAs), positively affect human health through supplying biologically active substances important for the metabolism (Janů et al., 2007; Bauman and Lock, 2010).

A world-wide important problem of Holstein dairy cows' breeding is the low level of their longevity (Zavadilová and Štípková, 2013) negatively affecting the lifelong total income to the cost sum rate per cow. This fact is a major reason for continuous longevity traits evaluation with subsequent inclusion in selection schemes (Přibyl et al., 2004; Šafus et al., 2005). Longevity improvement will cause increased parity average on farms as well as population level. Few studies have focused on the evaluation of the parity effect on milk FAs' proportion. Moreover, the limited findings described in these studies are not uniform (Thomson et al., 2000; Kelsey et al., 2003; Castillo et al., 2006). The mentioned relationships could be in direct conflict with EU legislation continuously emphasizing and requiring an increase in the quality, health, and safety of agriculture and food products (Velčovská and Sadílek, 2014). Based on analysed results, we can hypothesize that different dam age will significantly affect FAs' content and proportion in milk. With respect to potential prolonging of cows' productive life, it is important to clearly determine the effect of parity in relation to the health quality and safety of raw milk. Thus, the objective of this study was to determine and evaluate the effect of cow parity on the FAs' groups' proportion in Holstein cows' milk during the first phase of lactation, with an emphasis on its potential importance for consumer health.

Materials and methods

Animals and herd management

Holstein cows (n=25), 9 primiparous, 9 in the second, and 7 in the third and subsequent parities, were observed and sampled during the performed study. The average parity was 2.04 lactations and corresponded to the Holstein population average of parity recorded under the official milk recording system used in the Czech Republic. All the cows calved within one month, from the end of June to the end of July. The dams' daily milk yield during the period observed ranged from 11.34 to 47.2 L of milk with an average value of 28.60 L and standard deviation of 9.20 L. All the cows selected for observation were without reproduction and health disorders in previous lactations as well as during the evaluated lactation phase. Determinations of body condition scoring (BCS) and fat to protein ratio (FPR) were used for detection of and excluding metabolism intensity and the negative energy balance (NEB) effect on milk fatty acids' content and proportion. Cows' BCS was determined monthly in accordance with the methodology of linear description and type evaluation of Holstein cattle used in the Czech Republic using a body condition index with a 5-point scale with 0.25 point increments (Ferguson et al., 1994). BCS ranged from 1.5 to 3.75 points with an average of 2.69 and standard deviation 0.37. The cows were loose housed in a cubicle straw-bedded barn. All the animals were fed a total mixed ratio consisting of maize silage, alfalfa silage, straw, grass hay, alfalfa hay, concentrates, brewery draff, bakery waste, and mineral supplements. The ingredient composition of the diet corresponded to the level of the dams' individual daily milk yield. Feeding rations were completely balanced for energy, protein, and fat, as well as mineral and vitamin content, and consisted of the same components through the entire experimental period.

Milk sample collection and analysis

Milk sampling started exactly on the 7th day after calving of each cow. Milk samples were collected precisely at 7-day intervals during the first 17 weeks of lactation. A total of 17 samples were obtained per dam observed, that is, 425 milk samples in all. Two aliquot milk samples were collected from each cow in accordance with the ICAR methodology applied within the milk recording system in the Czech Republic. The first sample with a preservative was heated to 39±1 °C and used for basic milk components content determination (% of fat and protein) using Milkoscan 133B (N. Foss Electric; Denmark). FPR was subsequently determined from fat and protein % content. The second sample without a preservative was used for the extraction of fat and the determination of fatty acids' (FAs) content. The gravimetric (reference) method in accordance with EN ISO 1211 (2010) applied as a standard in the Czech Republic for milk fat extraction was used. The extract was obtained using a water-based solution of ammonia, ethanol, diethylether and petrolether. FAs' methyl esters were prepared by the potassium hydroxide catalysed methylation and extracted into heptane. Gas chromatography of FAs' methyl esters was performed using the Master GC (DANI Instruments S.p.A.; Italy) (split regime, FID detector) on a column with polyethylene glycol stationary phase (FameWax - 30 mm x 0.32 mm x 0.25 μm). Helium was used as the carrier gas at a flow rate of 5 mL.min^{-1}. The temperature programme used for GC was as follows: 50 °C (2 min), after which the temperature was increased to 230 °C at 10 °C.min^{-1} (8 min). The temperature of the detector was 220 °C. The content (mg.100 g^{-1}) of FAs' groups (SFAs and its components HCFAs and VFAs; UFAs and its components MUFAs and PUFAs) was observed. HCFAs' content included lauric, myristic and palmitic acid in accordance with Kontkanen et al. (2011). Volatile fatty acids' (VFA) contents were determined from the content of butyric, caproic, caprylic, and capric FAs in accordance with the study by Pešek et al. (2006). Subsequently, the proportions (%) of the six FAs' groups observed were determined and evaluated.

Statistical analysis

Microsoft Office Excel and SAS 9.3 (SAS/STAT® 9.3, 2011) were used for data processing and evaluating. The UNIVARIATE procedures were applied for descriptive statistics determination. The correlation coefficients determining the relationships among milk FAs' groups' content, BCS, fat content, FPR, and milk yield were evaluated using the CORR procedure. The MIXED procedure repeatability model was used for individual evaluations. The variance component covariance matrix was used for modelling of the lactation week effect within the repeatability model. Akaike Information Criterion was used for the best model determination. FAs' groups' (SFAs and its components HCFAs and VFAs; UFAs and its components MUFAs and PUFAs) proportion in the milk fat was evaluated by a designed model including the effect of parity, NEB, and regression on lactation week, as well as FPR. The dams were grouped into 3 classes according to parity (the 1st, 2nd, resp. 3rd and subsequent lactations). The effect of NEB was represented by two levels only (YES - within the NEB period, NO - after the NEB period) according to the BCS changes. A decrease in BCS meant classification within the NEB period, while balanced or increased BCS was labelled as after the NEB. According to BCS changes, the average length of NEB was 10.12 weeks. FPR regression added into the model clarified NEB determination more precisely, referring to BCS evaluation once a month. The Tukey-Kramer method was applied for comparison and evaluation of the differences in significance between least square means. The mixed model equation used for the evaluation was as follows:

$$Y_{ijk} = \mu + PAR_i + NEB_j + b_1{*}(WEEK) + b_2{*}(FPR) + e_{ijk}$$

where:

Y_{ijk} = dependent variable (SFAs and its components HCFAs, VFAs in %; UFAs and its components MUFAs and PUFAs in %; n = 425);

μ = mean value of dependent variable;

PAR_i = fixed effect of i^{th} parity of lactation (i = the 1st lactation, n = 153; the 2nd lactation, n = 153; the 3rd and subsequent lactations, n = 119);

NEB_j = fixed effect of j^{th} NEB period occurrence (j= YES - within NEB period, n = 242; NO - after NEB period, n = 183);

b_1*(WEEK) = regression in order of lactation week;

b_2*(FPR) = regression in milk fat to protein ratio on sampling day;

e_{ijk} = random error.

Significance levels P<0.05 and P<0.01 were used to evaluate the differences between groups.

Results and discussion

The average parity of cows observed achieved 2.04 lactations and corresponded with the average level of Holstein cows' parity in the Czech Republic. Fat proportion presented 3.80 % on the average with standard deviation 0.84. The average level of FPR determined was 1.21 with a standard deviation 0.29. The proportion of observed FAs' groups was detected at the levels as follows: SFAs 75.10 % and of them HCFAs 43.03 % and VFAs 20.43 %, while UFAs were 24.90 % and of them MUFAs 21.50 %

and PUFAs 3.39 %. The basic characteristics of the applied model described its suitability for the performed evaluation. The model repeatability ranged from 0.18 within VFAs' proportion evaluation to 0.38 in relation to SFAs' and UFAs' determination, when statistical significance of the entire model was $P<0.001$ in all evaluations as well as the effect of lactation week and FPR regression. NEB effect was significant under evaluation of all FAs' groups ($P<0.05$ to 0.001). Parity effect as the main factor evaluated significantly ($P<0.001$) affected the proportion of all FAs, except VFAs ($P=0.220$).

Milk FAs' contents and/or proportions are influenced by animal species, or rather breed (Pešek et al., 2005; Marounek et al., 2012), cows' genetic individuality (Soyeurt et al., 2006), year and season, as well as milking time (Toušová et al., 2013), lactation phase (Kirchnerová et al., 2013), nutrition level (Kupczyński et al., 2012), milk yield (Kay et al., 2005), NEB course (Ducháček et al., 2012a), and dams' health (Vacek et al., 2007). However, we observed only healthy Holstein dams, calved within one month, all sampled during the first 17 weeks, and the same milkings, as well as fed with a feed ration consisting of the same components in relation to their daily milk yield. The NEB effect usually occurring within the first lactation phase was eliminated by the statistical model used. Therefore, we assume that it is possible to exclude the effect of the factors mentioned.

SFAs are considered as harmful due to their relationship to plasma cholesterol (Bauman and Lock, 2010), primarily HCFAs as their component. A higher proportion of the HCFAs' group in blood causes the deposition of fat in the blood vessel walls and leads to atherosclerotic disease (Haug et al., 2007). Kirchnerová et al. (2013) determined a continuous increase in milk HCFAs' values, however, based on phenotype correlations only. On the other hand, this effect was excluded under our evaluation with respect to regression ïn lactation week incorporated into the designed model.

The results from the MIXED model for fixed effect of parity are given in Tables 1 and 2. Significantly ($P<0.01$) the highest value of SFAs was determined in cows with the 2nd parity compared to primiparous (+2.07 %) and the 3rd and subsequent lactations (+3.08 %). As well, the significantly ($P<0.01$) highest values of HCFAs were found at the beginning of the 2nd lactation (+2.67, resp. +2.94 %) in comparison with cows in the 1st, resp. 3rd and subsequent lactations. Although the highest value of VFAs was also detected in the 2nd lactation (21.49 %), the differences determined among observed parities were insignificant. Similarly to our findings, Bastin et al. (2013) found the highest SFAs' content in cows in the 2nd (+3.32 %) compared to the 1st lactation, respectively the 3rd and subsequent lactations (+0.10 %). However, they did not look for possible explanations. On the other hand, Rani et al. (2011) determined the highest values of SFAs, MUFAs, and PUFAs in cows of the Nguni rustical breed during the 3rd lactation. They explained this fact by the reaching of body maturity and therefore, by higher accessible body fat reserves usable for more intensive synthesis of milk components (Sevi et al., 2000). The Holstein breed observed in our study has a more intensive metabolism compared to rustical and/or primitive breeds. The more intensive the metabolism and production, the lower the robustness of the organism is in relation to the higher age of dairy cows. This relationship is directly documented by Holstein dams' average age in culling from the herd. Páchová et al. (2005) and Zavadilová and Štípková (2013) detected a decline of the average length of production life from 2.53 to almost only 2 lactations in relation to a 305-day milk yield increase from 6.662 kg of milk in 2004 to 8.047 kg in 2012.

Lower SFAs, respectively HCFAs and VFAs in the 1st as well as the 3rd and subsequent lactations indicate more intensive metabolism (Ducháček et al., 2012a; Ducháček et al., 2014). The results mentioned have a biological background. Primiparous cows undergo many physiological changes corresponding to the beginning of milk production, and consequently they have a considerably burdened metabolism. Exhaustion of the cows' organism is most frequently detected from the 3rd lactation due to continued increase of milk production and its maintenance up to the 5th lactation (Mellado et al., 2011). The processing and consumption of milk from primiparous cows as well as those in the 3rd lactation and older seem to be the most optimal as regards its nutrient and health quality. These dairy cows produced milk with a significantly lower content of SFAs and HCFAs ($P<0.05$).

UFAs and mainly its components PUFAs are the milk FAs beneficial for human nutrition and

Table 1. Effect of cow parity on proportion of milk saturated fatty acids (SFAs), hypercholesterolemic fatty acids (HCFAs), and volatile fatty acids (VFAs)

Factor	Level	SFAs (%)	HCFAs (%)	VFAs (%)
		LSM±SE	LSM±SE	LSM±SE
parity	1st lactation	74.51±0.394^{A}	41.81±0.315^{A}	20.25±0.572
	2nd lactation	76.58±0.413^{B}	44.48±0.330^{B}	21.49±0.600
	≥3rd lactation	73.50±0.453^{A}	41.54±0.361^{A}	20.24±0.657

LSM - least square means; SE - standard error of the least square means; A, B - different superscript letters confirm statistical significance of difference among rows at the P < 0.01 level

Table 2. Effect of cow parity on proportion of milk unsaturated (UFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs)

Factor	Level	UFAs (%)	MUFAs (%)	PUFAs (%)
		LSM±SE	LSM±SE	LSM±SE
parity	1st lactation	25.49±0.394^{A}	22.06±0.373^{A}	3.42±0.056^{a}
	2nd lactation	23.42±0.413^{B}	20.20±0.391^{B}	3.22±0.059^{b,A}
	≥3rd lactation	26.50±0.453^{A}	22.90±0.428^{A}	3.60±0.064^{B}

LSM - least square means; SE - standard error of the least square means; a, b; resp. A, B - different superscript letters confirm statistical significance of difference among rows at the P<0.05; resp. P<0.01 level

health. On the other hand, Hostens et al. (2013) mentioned that increasing the UFA content of milk should not be a goal as such when supplementing UFA to dairy cows, as higher bulk tank UFA are associated with worsened fertility results. The UFAs', MUFAs', and PUFAs' proportions showed the opposite results compared to SFAs' evaluation. Significantly (P<0.01-0.05) the lowest UFAs' (23.42 %), MUFAs' (20.20 %), and PUFAs' values (3.22 %) under the effect of parity were observed in cows in the 2nd lactation (Table 2). The highest values (P<0.01) for these FAs' groups were determined in cows in the 3rd and subsequent lactations.

On the contrary, Thomson et al. (2000) documented higher UFAs' values in Holstein primiparous cows compared to the 2nd and subsequent lactations. Bastin et al. (2013) described the highest MUFAs' level (+1.06 to +1.67 %) in the milk of primiparous as well. However, similar to our results, they determined the highest PUFAs' values (+2.26 to +4.52 %) in cows in the 3rd and subsequent lactations. Inconsistent results were detected by Secchiari et al. (2003), who did not find significant differences among observed parities. The above-mentioned disagreements can be caused by seasonal effect, different herd management, nutrition type and quality, as well as the methodology of individual observations, for example, by different groupings of dams according to parity or by less suitable statistical evaluation. Based on our results, it is possible to recommend consumption of milk from primiparous cows as well as those in the 3rd and subsequent lactation, because it contains a higher average MUFAs' and PUFAs' % proportion. A similar explanation is possible referring to SFAs' proportion. Ducháček et al. (2014) stated the significant effect of an organism energy status on FAs' content and % proportion. As mentioned above, the energy status of cows in the 2nd parity is the most balanced. Therefore, lower intensity of fat reserves' degradation simultaneously followed by better utilization of feed nutrients (Bauman and Griinari, 2001) along with subsequent decline of fat content in milk (Čejna and Chládek, 2005) occurred. UFAs' content in milk is desirable for consumer health, mainly according to conjugated linoleic acid content (Domagała et al., 2013) and other essential FAs (Innis, 2007) functioning in an organism as biologically active substances. A decrease in concentration of UFAs in milk during lactation, especially concentration of PUFAs, is a physiologically natural state, although undesirable as regards the quality of milk as a basic food (Gross et al., 2011). Simultaneously, increasing the milk UFA is not desirable because of negative correlation to dairy cows' fertility capabilities as described by Hostens et al. (2013). Concurrently, there are other food commodities which are valuable natural source of FAs for human diet (Staňková et al., 2013; Jokić et al., 2013). Therefore, we can suppose that a decrease in these milk FAs through the lactation period has a lower importance in comparison to HCFAs' content changes.

Conclusion

In general, cow's milk is characterised by high SFAs' content, with mainly HCFAs as the most detrimental. On the other hand, PUFAs as a component of UFAs are considered to be the healthiest FAs in milk. The lowest proportion of unhealthy HCFAs (-0.27 to -2.94 %) and simultaneously the highest proportion of healthy UFAs (+1.01 to 3.08 %) were detected in the milk of cows in the 3rd and subsequent lactations. An organism stressed by repeated lactations is subjected to more intensive and longer negative energy status, causing a lower proportion of SFAs, HCFAs and VFAs, as well as a higher content of UFAs, MUFAs and PUFAs. Thus, these dairy cows produce milk with a healthier composition of FAs. This fact emphasizes the necessity of prolongation of dairy cows' longevity from the point of view of milk quality as a basic animal product widely used for human nutrition. Breeding and selection of Holstein dairy cattle focusing on longevity are currently aiming mainly at increasing economic efficiency. However, positive changes in milk FAs' composition expressed by lower HCFAs and higher UFAs can be a simultaneous effect. The results are applicable within the framework of dairy farm management, selection, and breeding of dairy cattle breeds at the population level as well as in dairy plants processing raw cow's milk as a basic human food.

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Utjecaj rednog broja laktacije na omjer važnih zdravih masnih kiselina u sirovom mlijeku krava holstein pasmine

Sažetak

Cilj ovog istraživanja bio je utvrditi i procijeniti utjecaj rednog broja laktacije na udio pojedinih skupina masnih kiselina, u mlijeku holstein krava tijekom prve faze laktacije, s naglaskom na njihovu potencijalnu važnost za zdravlje potrošača. Ukupno 25 holstein krava, devet prvotelkinja, devet u drugoj, i sedam u trećoj i naknadnim laktacijama, promatrane su i uzorkovane u sedmodnevnim intervalima tijekom prvih 17 tjedana laktacije. Utvrđivan je udio zasićenih (unutar te skupine hiperkolesterolemičnih i hlapivih masnih kiselina) te nezasićenih (unutar te skupine mononezasićenih i polinezasićenih) masnih kiselina u uzorcima mliječne masti (n=425). Utjecaj rednog broja laktacije i negativne energetske bilance, kao i regresija, na tjedne laktacije i omjer masti i proteina procijenjeni su pomoću SAS 9.3. Značajno (P<0,01) manji udio po zdravlje nepovoljnih hiperkolesterolemičnih masnih kiselina utvrđen je u prvotelkinja (-2,67 %) i onih u 3. i naknadnim laktacijama (-2,94 %) u odnosu na krave u drugoj laktaciji, kao i istodobno veći udio po zdravlje povoljnih nezasićenih masnih kiselina (2,07 %, odnosno 3,08 %). Utvrđena veza objašnjava se stresom organizma prouzrokovanim početkom proizvodnje mlijeka i održavanjem te proizvodnje tijekom kasnijih laktacija. Dakle, općenito dulji životni vijek mliječnih krava može utjecati na kvalitetu sirovog mlijeka, osobito s obzirom na sastav masnih kiselina.

Ključne riječi: govedo, dugovječnost, proizvodnja mlijeka, kvaliteta mlijeka, sastav mliječne masti

References

1. Bastin, C., Soyeurt, H., Gengler, N. (2013): Genetic parameters of milk production traits and fatty acid contents in milk for Holstein cows in parity 1-3. *Journal of Animal Breeding and Genetics 130* (2), 118-127.
2. Bašić, Z., Božanić, R., Konjačić, M., Đermadi, J., Antunac, N., Volarić, V. (2012): Chemical and microbiological farm milk quality determination in three Croatian regions. *Mljekarstvo 62* (4), 251-260.

3. Bauman, D.E., Griinari, J.M. (2001): Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livestock Production Science 70* (1), 15-29.
4. Bauman, D.E., Lock, A.L. (2010): Milk fatty acid composition: challenges and opportunities related to human health, XXVI World Buiatrics congress, 14-18 Nov 2010, Santiago, Chile, 278-289.
5. Castillo, A.R., Taverna, M.A., Paez, R.R., Cuatrin, A., Colombatto, D., Bargo, F., García, M.S., García, P.T., Chavez, M., Beaulieu, A.D., Drackley, J.K. (2006): Fatty acid composition of milk from dairy cows fed fresh lucerne based diets. *Animal Feed Science and Technology 131* (3-4), 241-254.
6. Čejna, V., Chládek, G. (2005): The importance of monitoring changes in milk fat to milk protein ratio in Holstein cows during lactation. *Journal of Central European Agriculture 6* (4), 539-546.
7. Domagała, J., Pluta-Kubica, A., Pustkowiak, H. (2013): Changes in conjugated linoleic acid content in Emmental-type cheese during manufacturing. *Czech Journal of Food Science 31* (5), 432-437.
8. Ducháček, J., Vacek, M., Stádník, L., Beran, J., Okrouhlá, M. (2012a): Changes in milk fatty acid composition in relation to indicators of energy balance in Holstein cows. *Acta universitatis agriculaturae et silviculturae Mendelianae Brunensis 60* (1), 29-38.
9. Ducháček, J., Stádník, L., Beran, J., Okrouhlá, M. (2012b): The relationship between fatty acid and citric acid concentrations in milk from Holstein cows during the period of negative energy balance. *Journal of Central European Agriculture 13* (4), 615-630.
10. Ducháček, J., Stádník, L., Beran, J., Okrouhlá, M. (2013): Comparison of different sorting of fatty acids in bovine milk in relation to body condition of Czech Fleckvieh dairy cows. *Journal of Central European Agriculture 14* (3), 137-148.
11. Ducháček, J., Stádník, L., Ptáček, M., Beran, J., Okrouhlá, M., Čítek, J., Stupka, R. (2014): Effect of Cow Energy Status on Hypercholesterolemic Fatty Acids Proportion in Raw Milk. *Czech Journal of Food Science 32* (in press, http://www.agriculturejournals.cz/web/cjfs.htm?type=futureArticles).
12. EN ISO 1211 (ČSN 570534) (2010): Milk – Determination of fat content - Gravimetric method (Reference method), European standard EN ISO 1211:2010.
13. Ferguson, J.D., Galligano, D.T., Thomsen, N. (1994): Principal descriptors of body condition score in Holstein cows. *Journal of Dairy Science 77* (9), 2695-2703.
14. Gergovska, Z., Miteva, T., Angelova, T., Yordanova, D., Mitev, J. (2012): Relation of milking temperament and milk yield in Holstein and Brown Swiss cows. *Bulgarian Journal of Agriculture Science 18* (5), 771-777.
15. Gross, J., van Dorland, H.A., Bruckmaier, R.M., Schwarz, F.J. (2011): Milk fatty acid profile related to energy balance in dairy cows, *Journal of Dairy Research 78* (4), 479-488.
16. Haug, A., Høstmark, A.T., Harstad, O.M. (2007): Bovine milk in human nutrition - a review. *Lipids in Health and Disease 6* (25), 1-16.
17. Hostens, M., Fievez, V., Leroy, J.L.M.R., van de Burgwal, E.J., Van Ranst, B., Vlaeminck, B., Opsomer, G. (2013): Milk Fat Saturation and Reproductive Performance in Dairy Cattle. *Animal Reproduction Science 141*, 116-123.
18. Innis, S.M. (2007): Essential fatty acids in infant nutrition: lessons and limitations from animal studies in relation to studies on infant fatty acid requirements. *The American Journal of Clinical Nutrition 71* (1 Suppl), 238S-244S.
19. Ivanova, T., Metodiev, N., Raicheva, E. (2013): Effect of the genealogic line on milk production and prolificacy of the ewes from Synthetic population Bulgarian milk. *Bulgarian Journal of Agriculture Science 19* (1), 158-162.
20. Ivkić, Z., Špehar, M., Bulić, V., Mijić, P., Ivanković, A., Solić, D. (2012): Estimation of genetic parameters and environmental effects on somatic cell count in Simmental and Holstein breeds. *Mljekarstvo 62* (2), 143-150.
21. Janů, L., Hanuš, O., Macek, A., Zajíčková, I., Genčurová, V., Kopecký, J. (2007): Fatty acids and mineral elements in bulk milk of Holstein and Czech Spotted cattle according to feeding season. *Folia Veterinaria 51*, 19-25.
22. Jensen, R.G. (2002): The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science 85*, 295-350.
23. Jokić, S., Suder, R., Svilović, S., Vidović, S., Bilić, M., Velić, D., Jurković, V. (2013): Fatty acid composition of oil obtained from Soyabeans by extraction with supercritical Carbon Dioxide. *Czech Journal of Food Science 31* (2), 116-125.
24. Kadlecová, V., Němečková, D., Ječmínková, K., Stádník, L. (2014): The effect of polymorphism in the DGAT1 gene on energy balance and milk production traits in primiparous Holstein cows during the first six months of lactation. *Bulgarian Journal of Animal Science 20* (1), in press.
25. Kay, J.K., Weber, W.J., Moore, C.E., Bauman, D.E., Hansen, L.B., Chester-Jones, H., Crooker, B.A., Baumgard, L.H. (2005): Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. *Journal of Dairy Science 88* (11), 3886-3893.
26. Kelsey, J.A., Corl, B.A., Collier, R.J., Bauman, D.E. (2003): The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *Journal of Dairy Science 86* (8), 2588-2597.
27. Kirchnerová, K., Foltys, V., Špička, J. (2013): Impact of lactation stage on milk fat fatty acids profile in grazing dairy cows. *Journal of Microbiology, Biotechnology and Food Sciences 2*, 1164-1174.
28. Klir, Ž., Antunović, Z., Novoselec, J. (2012): Utjecaj hranidbe koza na sadržaj masnih kiselina u mlijeku. *Mljekarstvo 62* (4), 231-240.
29. Kontkanen, H., Rokka, S., Kemppinen, A., Miettinen, H., Hellström, J., Kruus, K., Marnila, P., Alatossava, T., Korhonen, H. (2011): Enzymatic and physical modification of milk fat: A review. *International Dairy Journal 21* (1), 3-13.

30. Kupczyński, R., Kuczaj, M., Szołtysik, M., Stefaniak, T. (2012): Influence of fish oil, palm oil and glycerol on milk fatty acid composition and metabolism in cows during early lactation. *Archiv für Tierzucht* 55 (6), 540-551.

31. Marounek, M., Pavlata, L., Mišurová, L., Volek, Z., Dvořák, R. (2012): Changes in the composition of goat colostrum and milk fatty acids during the first month of lactation. *Czech Journal of Animal Science 57* (1), 28-33.

32. Mellado, M., Antonio-Chirino, E., Meza-Herrera, C., Veliz, F.G., Arevalo, J.R., Mellado, J., de Santiago, A. (2011): Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin. *Journal of Dairy Science 94* (9), 4524-4530.

33. Páchová, E., Zavadilová, L., Sölkner, J. (2005): Genetic evaluation of the length of productive life in Holstein cattle in the Czech Republic. *Czech Journal of Animal Science 50* (11), 493-498.

34. Pešek, M., Špička, J., Samková, E. (2005): Comparison of fatty acid composition in milk fat of Czech Pied cattle and Holstein cattle. *Czech Journal of Animal Science 50* (3), 122-128.

35. Pešek, M., Samková, E., Špička, J. (2006): Fatty acids and composition of their important groups in milk fat of Czech Pied cattle. *Czech Journal of Animal Science 51* (5), 181-188.

36. Přibyl, J., Šafus, P., Štípková, M., Stádník, L., Čermák, V. (2004): Selection index for bulls of Holstein cattle in the Czech Republic. *Czech Journal of Animal Science 49* (6), 244-256.

37. Rani, Z.T., Chimonyo, M., Hugo, A., Marume, U., Muchenje, V. (2011): Effect of parity on the proximate composition and fatty acid profile of milk from Hguni cattle grazing on natural pasture. *African Journal of Biotechnology 10* (43), 8647-8653.

38. SAS Institute Inc. (2011): SAS/STAT® 9.3 User's Guide. Cary, NC: SAS Institute Inc.

39. Secchiari, P., Mele, M., Serra, A., Buccioni, A., Paoletti, F., Antongiovanni, M. (2003): Effect of breed, parity and stage of lactation on milk conjugated linoleic acid content in Italian Friesian and Reggiana cows. *Italian Journal of Animal Science 2* (Suppl.1), 269-271.

40. Sevi, A., Taibi, L., Albenzio, M., Muscio, A., Annicchiarico, G. (2000): Effect of parity on milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. *Small Ruminant Research 37* (1-2), 99-107.

41. Soyeurt, V., Dardenne, P., Gillon, A., Croquet, C., Vanderick, S., Mayeres, P., Bertozzi, C., Gengler, N. (2006): Variation in fatty acid content of milk and milk fat within and across breeds. *Journal of Dairy Science 89* (12), 4858-4865.

42. Stádník, L., Louda, F. (1999): The effect of genetic parameters of sire in France on the performance and reproduction of daughters imported to the Czech Republic and calving here. *Czech Journal of Animal Science 44* (10), 433-439.

43. Stádník, L., Louda, F., Bezdíček, J., Ježková, A. Rákos, M. (2010): Changes in teat parameters caused by milking and their recovery to their initial size. *Archiv für Tierzucht 53* (6), 650-662.

44. Staňková, B., Kremmyda, L.S., Tvrzická, E., Žák, A. (2013): Fatty Acid Composition of Commercially Available Nutrition Supplements. *Czech Journal of Food Science 31* (3), 241-248.

45. Szencziová, I., Strapák, P., Stádník, L., Ducháček, J., Beran, J. (2013): Relationship of udder and teat morphology to milking characteristics and udder health determined by ultrasonographic examinations in dairy cows. *Annals of Animal Science 13* (4), 783-795.

46. Šafus, P., Štípková, M., Stádník, L., Přibyl, J., Čermák, V. (2005): Sub-indexes for bulls of Holstein breed in the Czech Republic. *Czech Journal of Animal Science 50* (6), 254-265.

47. Thomson, N.A., van der Poel, W., Peterson, S.W. (2000): Seasonal variation of the fatty acid composition of milk fat from Friesian cows grazing pasture. *Proceedings of the New Zealand Society of Animal Production 60*, 314-317.

48. Toušová, R., Stádník, L., Ducháček, J. (2013): Effect of season and the time of milking on spontaneous and induced lipolysis in bovine milk fat. *Czech Journal of Food Science 31* (1), 20-26.

49. Vacek, M., Stádník, L. Štípková, M. (2007): Relationships between the incidence of health disorders and the reproduction traits of Holstein cows in the Czech Republic. *Czech Journal of Animal Science 52* (8), 227-235.

50. Velčovská, S., Sadílek, T. (2014): Analysis of quality labels included in European Union quality schemes. *Czech Journal of Food Science 32* (2), in press.

51. Zamberlin, Š., Antunac, N., Havranek, J., Samaržija, D. (2012): Mineral elements in milk and dairy products. *Mljekarstvo 62* (2), 111-125.

52. Zavadilová, L., Štípková, M. (2013): Effect of age at calving on longevity and fertility traits for Holstein cattle. *Czech Journal of Animal Science 58* (2), 47-57.