Effects of Season and Time of Milking on Spontaneous and Induced Lipolysis in Bovine Milk Fat

Renáta TOUŠOVÁ, Luděk STÁDNÍK and Jaromír DUCHÁČEK

Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Sources, Czech University of Life Sciences Prague, Prague-Suchdol, Czech Republic

Abstract

Toušová R., Stádník L., Ducháček J. (2013): **Effects of season and time of milking on spontaneous and induced lipolysis in bovine milk fat**. Czech J. Food Sci., **31**: 20–26.

The effects were evaluated of different factors on the level of spontaneous (SPO) and induced (IND) lipolysis as defined by the content of free fatty acids (FFA) in milk. Milk samples were collected at monthly intervals throughout the year from both morning and evening milkings either individually in a milking parlour (SPO; n = 10) or from the bulk tank (IND; n = 10). The data were analysed using SAS 9.1. More intensive SPO was observed from March to May with higher FFA contents (+0.034 to +0.523 mmol/100 g of fat; $P < 0.05–0.01$), and also from September to November (+0.077 to +0.292 mmol/100 g of fat; $P < 0.05$). More intensive SPO was also detected in the evening milk than in that coming from morning milking (+0.062 to +0.556 mmol/100 g of fat; $P < 0.05–0.01$). SPO measured immediately after milking was affected by the season and time of milking. The content of FFA characterising IND in bulk milk (0.33–1.10 mmol/100 g of fat) was higher ($P < 0.05–0.001$) than that due to SPO in individual samples (0.21–0.86 mmol/100 g of fat), especially in those from evening milking compared to morning milking (+0.10 to +0.47 vs. +0.12 to +0.22 mmol/100 g of fat; $P < 0.05–0.001$).

Keywords: dairy cow; free fatty acid; lipolysis; milking; processing

Milk fat is one of the principal milk solids, and its content considerably changes throughout the entire lactation period and also during a single day (Poláková *et al.* 2010). Milk fat consists of triacylglycerol, phospholipids, non-esterified fatty acids, and glycerol. In fresh milk, 99% of fatty acids are esterified to triacylglycerol, and their composition influences the nutritive and technological properties of milk and milk fat (Hanuš *et al.* 2010). A certain level of lipolytic activity caused by lipoprotein lipase occurs in each bovine milk sample. It results in the formation of free fatty acids, diacylglycerols, monoacylglycerols, and glycerol (Deeth 2006). Lipolysis of triacylglycerols to glycerol and free fatty acids (FFA) may lead to undesirable modifications in milk and dairy products. High quality raw milk, even after 24 h of storage including cooling and stirring, should not contain more than 1 mmol FFA per 100 g of fat. The amount of FFA depends on the extent of the damage to the fat globule membrane, and the value of 1.5 mmol/l of milk is considered as critical (Deeth 2006). Three different types of fat lipolysis may occur in milk depending on different factors: spontaneous, induced (Forman 1984), and microbial (Cempírková & Mikulová 2009). Spontaneous lipolysis is caused by the activity of milk lipases, and its intensity as well as milk fatty

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project MSM 6046070901, S Grant of MSMT CR, and by the Ministry of Agriculture of the Czech Republic, Project No. QH91270.

acid composition is influenced by the stage of lactation (González-Martín *et al.* 2009), diet type and composition (Cieslak *et al.* 2010; Kalač & Samková 2010), and energy status (Hanuš *et al.* 2010). Induced lipolysis is the result of mechanical damage to the fat globule membranes arising during milking, milk transport, storage, and processing, and is followed by the contact of free fat with milk lipases (Quattar *et al.* 2004). The level of microbial lipolysis depends on the incidence of lipolytic bacteria. Especially psychrotrophic microorganisms are considered dangerous, as they are able to reproduce even at storage temperatures (Cempírková & Mikulová 2009). Individual differences exist in the susceptibility to spontaneous lipolysis (Miller & Puhan 1985). Most lipolytic changes in chilled milk occur during the first 24 hours. It was reported that milk with a content of FFA higher than 1.5 mmol/l is not acceptable for human consumption (IDF 1987). Negative energy balance is another factor increasing the content of FFA. High milk yields and a limited ability of feed intake in early lactation trigger the process of lipomobilisation and the release of energy from the body fat reserves (Gonzáles *et al.* 2011). As a result, the contents of fat and FFA increase both in blood and milk (Kulig *et al.* 2010). Therefore, the content of FFA may indicate the health status of dairy cows (Hanuš *et al.* 2004). Under negative energy balance or low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of fatty acids in their nonesterified forms (NEFA, resp. FFA) into the bloodstream (Nelson & Cox 2000). Thus, the concentration of NEFA may reach a value detrimental to the health status of cows both prepartum (Poláková *et al.* 2010) and postpartum (Herdt 2007). A wide range of dairy cows' production diseases are treated using antibiotics which affect the final quality of raw milk as well (Navrátilová *et al.* 2011). Based on the facts mentioned above, we assume that differences in FFA contents can be detected among animals as well as among bulk tank samples collected in different seasons and milking times. It can also be assumed that differences exist in total FFA content depending on the intensity of spontaneous lipolysis in milk samples collected in a milking parlour, and that the FFA content will increase as a result of induced lipolysis due to mechanical stress imposed on milk during pumping, storage, cooling, and stirring in a tank. Little information is available on the content of FFA as affected by the season. In addition, sufficient data describing the lipolysis intensity in milk obtained at different milking times are entirely lacking. Therefore, the objective of this study was to determine the effects of season and the time of milking on the extent of spontaneous and induced lipolysis based on the contents of FFA in milk samples collected either from individual animals or from a bulk tank.

MATERIAL AND METHODS

Milk samples were collected from Holstein cows at monthly intervals throughout the year. The cows were kept in a loose-housing system on a slatted floor, and the manure was removed using a v-shaped scraper. The winter season diet consisted of 23 kg of maize silage, 6.5 kg of clover/grass silage, 6.2 kg bean-clover pellets, 1 kg of peas GPS, 1 kg of beans GPS, 2 kg of wheat straw, and the concentrate mixture digestible nitrogen substances (DOPS 121 g/kg, NEL 611.82 MJ/kg, fibre 58 g/kg, Ca 4.6 g/kg, and P 7.2 g/kg). The summer diet consisted of 35 kg of clover/grass mixture, 10 kg of maize silage, 2.5 kg of hay, 2 kg of wheat straw, and the concentrate mixture mentioned above. The transition periods for gradual diet changes were March to April and October to November. A carousel parlour was used for milking, and milk was stored in a 5000 l tank. The milk samples were collected from ten cows having calved in January. The parity of these cows was similar to that of the entire herd, 3 cows in the 1st lactation, 4 cows in the 2nd lactation, and 3 cows in the 3rd and subsequent lactations. Milk of all cows was checked by NK test and no symptoms of mastitis or high level of somatic cells were determined during the observation. Daily milk yield of the evaluated cows was in the range from 6.4 kg to 39.5 kg with the average value 18.57 kg and standard deviation 6.72. Fat content in the individual milk samples ranged from 2.85% (s_d = 0.610) to 4.24% (s_d = 0.367) in the morning milk and from 3.06% (s_d = 0.387) to 4.36% (s_d = 0.339) in the evening milk during the whole period observed. Milks from morning and evening milkings were sampled directly, in the parlour with the aim of determining the intensity of spontaneous lipolysis caused by the action of milk lipases. In addition, bulk milk samples (n = 10) were also collected from tanks 1 h after milking to measure the level of induced lipolysis, which

results from mechanical damage to fat globule membranes, thus making milk fat more accessible to lipases. The evening milk samples were stored in refrigerator at 6°C during the night and analysed together with the morning samples collected next day. The length of the period between the collection and analyses was 12 h with the evening samples and 2 h with the morning samples. The only one difference was due to the storage of the evening samples, however, the methodology of sampling, manipulation, transportation, and analyses was completely the same for both the morning and evening samples. The milk samples met all the microbiological requirements given by the quality standard, and significant differences were detected neither in total bacteria nor in psychrotrophic bacteria counts. Similar milking conditions were secured during the experimental period as each cow evaluated always entered the same parlour stall equipped with the same milking machine. The content of FFA was analysed using the Milcoscan FT 6000 apparatus (Foss, Hillerød, Denmark) based on infrared spectrophotometry. The following statistics were used to evaluate the magnitude and significance of differences between the dependent variables: arithmetic mean, standard deviation, and *P* value of statistical significance. The pair *t*-test was used to compare the differences at $P < 0.01$ and $P < 0.05$ levels of statistical significance. The calculations were performed using SAS 9.1 (SAS 2004).

RESULTS AND DISCUSSION

The content of FFA was determined in a total of 220 milk samples. Table 1 shows the development of spontaneous lipolysis demonstrated on the basis of average FFA contents from morning and evening milkings in different months of the experimental period. However, lipolysis may sometimes occur already during the process of milking due to rising and frothing of milk in milk lines and therefore, it is not always possible to distinguish between spontaneous and induced lipolysis (Evers *et al.* 2001). The milk from certain cows is particularly susceptible to spontaneous lipolysis, and the content of FFA may reach as much as 10 mmol/l (Deeth & Fitz-Gerald 1995). Such high levels were not, however, observed in our study. The milk from some other cows can be partially resistant to spontaneous lipolysis with the level of FFA less than 0.5 mmol/l. The average contents of FFA range from 0.24 to 1.16 mmol/100 g of fat depending on the diet, breed, and individual cows (Abeni *et al.* 2005). The levels of spontaneous lipolysis observed in our study (0.10–1.10 mmol/100 g of fat) were in agreement with those reported by other authors.

Compared to other months, higher FFA contents in morning milking samples were observed in May (+0.072 to +0.453 mmol/100 g of fat; $P < 0.05$–0.01), compared to evening milking samples in March and April (+0.034 to +0.523 mmol/100 g of fat; $P < 0.05$) (Table 1). Also, FFA contents in milks

Table 1. Mean free fatty acid contents (mmol/100 g of fat) in morning and evening milk samples of 10 dairy cows (spontaneous lipolysis of milk fat)

Month	Morning	Evening	*P*
I (A)	0.400 ± 0.18^{DjK}	0.527 ± 0.16^{Cgh}	*
II (B)	0.327 ± 0.16^{Egjk}	0.445 ± 0.13^{Cd}	N
III (C)	0.306 ± 0.11^{dEgjk}	0.862 ± 0.34^{efGHIjK}	**
IV (D)	0.197 ± 0.08^{EFGhIJK}	0.665 ± 0.26^{fGH}	**
V (E)	0.650 ± 0.26^{fH}	0.491 ± 0.22	N
VI (F)	0.394 ± 0.09^{k}	0.467 ± 0.11^{gh}	N
VII (G)	0.479 ± 0.16^{H}	0.339 ± 0.10^{iJk}	N
VIII (H)	0.307 ± 0.08^{jk}	0.369 ± 0.07^{iJk}	*
IX (I)	0.442 ± 0.18^{jk}	0.578 ± 0.24^{j}	*
X (J)	0.578 ± 0.24	0.631 ± 0.22	N
XI (K)	0.506 ± 0.15	0.544 ± 0.19	N

month – month of lactation/calendar month; upperscript letters means significant difference among rows – a, b, c, d, e , f, g, h, i, j, k = $P < 0.05$; A, B, C, D, E, F, G, H, I, J, K = $P < 0.01$; stars means significant difference between columns – *$P < 0.05$, **$P < 0.01$, N = $P > 0.05$

coming from both times of milking were higher in October and November than in the summer months (+0.077 to +0.292 mmol/100 g of fat; $P < 0.05$). These results are in agreement with those of Hanuš *et al.* (2008), who reported that higher FFA contents in milk, were frequently caused by metabolic disorders of cows. In our study, such disorders were due to the deteriorated feed quality at the end of both winter and summer periods, and due to the problems in the transition periods from winter to summer and from summer to winter diets. Significant differences in morning FFA contents were observed between April and most other months (–0.109 to –0.453 mmol/100 g of fat; $P < 0.05$–0.01). Significant differences were also detected between May and February, March, and April ($P < 0.01$). Morning concentrations of FFA observed in October and November differed from those found in January, February, March, April, August, and September ($P < 0.05$–0.01). Compared to the other months, the highest evening FFA content was observed in March ($P < 0.05$–0.01). The evening FFA contents were lower ($P < 0.05$–0.01) in July and August compared to those in January, March, April, June, September, October, and November. On the contrary, higher FFA contents in milk were previously detected during the pasture season compared to the rest of the year (Frelich *et al.* 2009). However, the effect of the time of milking was not tested in that study. Our results confirm the seasonal character of FFA contents, with the most distinct changes observed in March and April, thus in the transition period from winter to summer diets. The contents of FFA in both morning and evening milk samples were higher from September to November. Milk fat composition is mainly affected by the diet (Samková *et al.* 2009; Dai *et al.* 2011). Our results are also in agreement with the study by Kon and Saito (1997), who reported that particularly cows in late lactation may be endangered by spontaneous lipolysis due to low milk yields and less intensive diet. It was suggested that the increased milk content of FFA in late lactation is associated with a poor energy intake at the end of the pasture season (September to November). Body fat deposits are mobilised, and FFA as a source of energy are released into the blood and transported into milk (Kulig *et al.* 2010). The fat content and the fat/protein ratio in milk therefore belong among the most important indicators of energy balance in animals (Sojková *et al.* 2010; Ducháček *et al.* 2012). The results concerning the energy status of animals also confirm the conclusions by Hanuš *et al.* (2008).

The differences in FFA contents indicating the level of spontaneous lipolysis were tested between the milk samples from morning and evening milkings of 10 cows (Table 1). Significantly higher FFA contents in milk from evening milkings ($P < 0.05$–0.01) were detected only in January, March, April, August, and September (+0.062 to +0.556 mmol/100 g of fat). In most other months,

Table 2. Mean free fatty acid contents (mmol/100 g of fat) in morning and evening milk samples from selected dairy cows ($n = 10$; spontaneous lipolysis – SPO) and from the bulk tank ($n = 10$; induced lipolysis – IND)

Month	Morning		Evening	
	SPO	IND	SPO	IND
I	0.400 ± 0.18	0.33 ± 0.02	0.527 ± 0.16	0.65 ± 0.07^{A}
II	0.327 ± 0.16	0.48 ± 0.03^{A}	0.445 ± 0.13	0.70 ± 0.07^{C}
III	0.306 ± 0.11	0.49 ± 0.04^{C}	0.862 ± 0.34	0.64 ± 0.11
IV	0.197 ± 0.08	0.31 ± 0.03^{B}	0.665 ± 0.26	0.77 ± 0.09
V	0.650 ± 0.26	0.72 ± 0.06	0.491 ± 0.22	0.66 ± 0.05^{A}
VI	0.394 ± 0.09	0.44 ± 0.03	0.467 ± 0.11	0.61 ± 0.03^{B}
VII	0.479 ± 0.16	0.55 ± 0.04	0.339 ± 0.10	0.44 ± 0.02^{B}
VIII	0.307 ± 0.08	0.43 ± 0.03^{C}	0.369 ± 0.07	0.49 ± 0.02^{C}
IX	0.442 ± 0.18	0.49 ± 0.05	0.578 ± 0.24	0.77 ± 0.06^{A}
X	0.578 ± 0.24	0.80 ± 0.08^{A}	0.631 ± 0.22	1.10 ± 0.13^{C}
XI	0.506 ± 0.15	0.52 ± 0.05	0.544 ± 0.19	0.71 ± 0.05^{A}

month – month of lactation/calendar month; A = $P < 0.05$, B = $P < 0.01$, C = $P < 0.001$ between columns

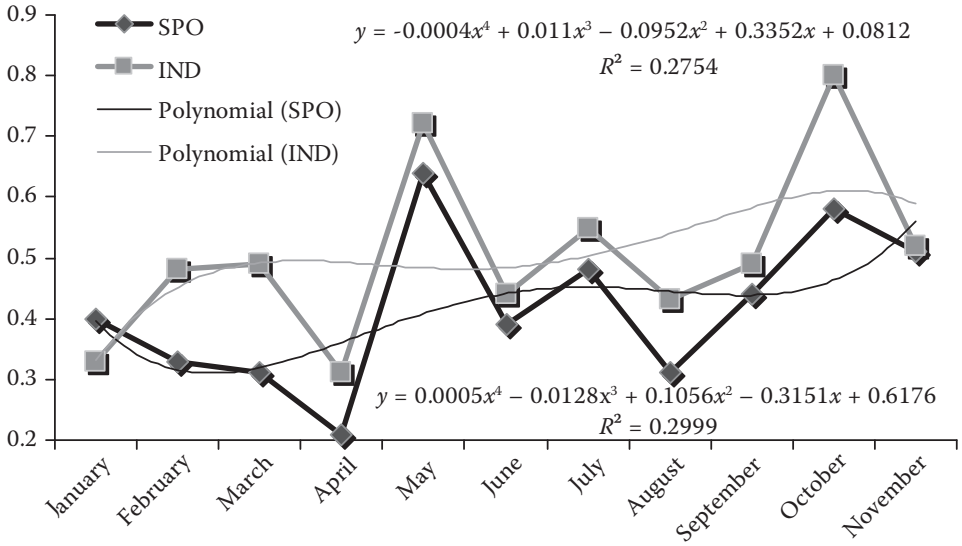

Figure 1. Development of mean free fatty acid contents (mmol/100 g of fat) in the morning milk from selected dairy cows (n = 10; spontaneous lipolysis – SPO) and from the bulk tank (n = 10; induced lipolysis – IND)

FFA contents were also higher in the evening milk, but the differences lacked statistical significance ($P > 0.05$). Previous studies were mostly directed at the effects of the lactation stage or season on different types of lipolysis in bulk milk, and the information on differences between morning and evening milkings is thus not available.

FFA contents in morning and evening milks from 10 cows as spontaneous lipolysis indicators were compared to those determined in bulk milk considered to be the indicators of induced lipolysis (Table 2). The average FFA content in bulk milk (0.33–1.10 mmol/100 g of fat) is higher than that in the samples coming from individual cows (0.21–0.86 mmol/100 g of fat), particularly in the evening compared to morning milk (+0.10 to +0.47 vs. +0.12 to 0.22 mmol/100 g of fat; $P <$ 0.05–0.001). Significant differences in evening FFA contents were detected between all the months except for March and April, whereas morning FFA contents differed in February, March, April, August, and October. October contents of FFA in bulk milk exceeded 1.0 mmol/100 g of fat thus indicating its deteriorated quality, however, they were below the critical value of 1.5 mmol/l of milk (Deeth 2006). As the time interval between the end of milking and the analyses performed was always the same, it is evident that the higher FFA contents in bulk milk were due to induced lipolysis as a result of milk fat globule disruption and exposure of the lipid substrate to the action of lipase. This occurred during the process of pumping and transport of milk to the bulk tank, which was followed by its stirring, cooling, and storage (Quattar *et al.* 2004; Evers 2004). A similar FFA content increase from 0.68 to 1.21 mmol/100 g of fat corresponding to our results was reported earlier (Wiking *et al.* 2006). However, the differences

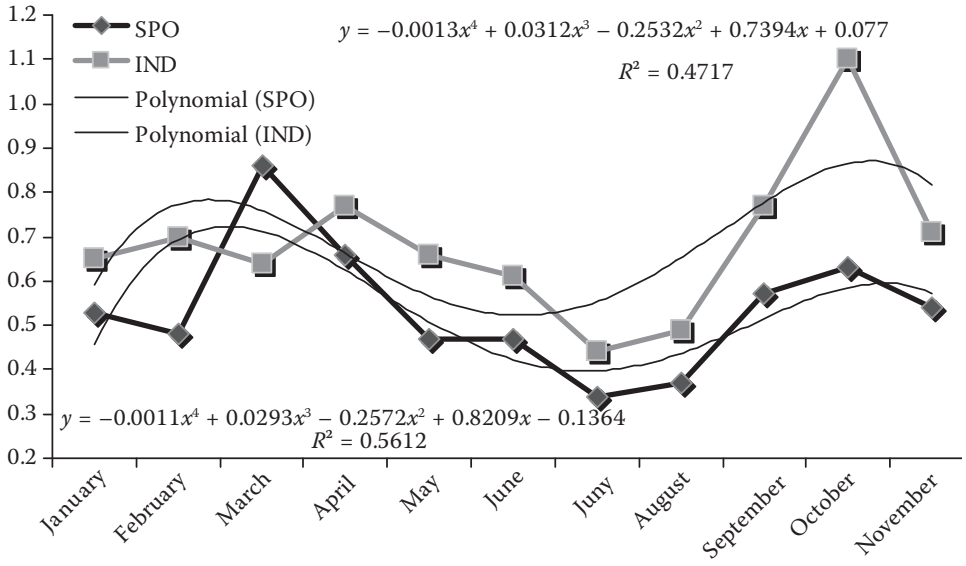

Figure 2. Development of mean free fatty acid contents (mmol/100 g of fat) in the evening milk from selected dairy cows (n = 10; spontaneous lipolysis – SPO) and from the bulk tank (n = 10; induced lipolysis – IND)

between the morning and evening FFA contents were not accounted for in previous studies.

Figures 1 and 2 show the development of FFA contents in the morning and evening milks as well as polynomial regression equations including reliability values. The reliability of the trend function was higher with the evening milking, which may have been related to the elevated contents of solid milk components, especially milk fat, in both individual and bulk samples (+0.04 to +0.41 g/1 and +0.14 to 0.44 g/1, respectively). However, this fact was not confirmed by Pearson correlation coefficients calculated between the fat and FFA contents. Also, the pattern of the trend functions was similar with the individual and bulk samples collected during evening milking. The same pattern of the trend functions and the higher reliabilities (R^2 higher by +0.1963 to +0.2613) confirm the higher informative value of higher FFA contents in both individual and bulk samples of the milk collected during evening milking.

CONCLUSIONS

It was confirmed that the content of FFA and thus the level of spontaneous lipolysis was affected by the individuality of the cow. Induced lipolysis characterised by increased contents of FFA in bulk milk samples occurred due to the damage to milk fat globules during milk processing (transport, cooling, stirring, and storage) already at the farm level. FFA content differences existed between bulk milk and individual milk samples collected from 10 cows, and, in addition, they were also affected by the calendar month and diet intensity. Higher FFA contents resulting from both spontaneous and induced lipolysis were detected in evening milk samples.

The results of this study indicate the need for further investigation of milk lipolysis with regard to its relationship to negative energy balance, as this is considered the main cause of fertility and health problems in dairy cows. The FFA content in milk was higher than 1.0 mmol/100 g of fat in several months even before the transport of milk from the farm and therefore, the result of this study may raise the necessity of determining the FFA content as an indicator of milk quality at both farm and dairy plant levels.

Acknowledgements. We thank Mrs. Lois Russell for her editorial help with the manuscript.

References

Abeni F., Degano L., Calza F., Giangiacomo R., Pirlo G. (2005): Milk quality and automatic milking: Fat globule size, natural creaming, and lipolysis. Journal of Dairy Science, **88**: 3519–3529.

Cempírková R., Mikulová M. (2009): Incidence of psychrotrophic lipolytic bakteria in cow's raw milk. Czech Journal of Animal Science, **54**: 65–73.

Cieslak A., Kowalczyk J. Czauderna M., Potkanski A., Szumacher-Strabel M. (2010): Enhancing unsaturated fatty acids in ewe's milk by feeding rapeseed or linseed oil. Czech Journal of Animal Science, **55**: 496–504.

Dai X.J., Wang C., Zhu Q. (2011): Milk performance of dairy cows supplemented with rapeseed oil, peanut oil and sunflower seed oil. Czech Journal of Animal Science, **56**: 181–191.

Deeth H.C. (2006): Lipoprotein lipase and lipolysis in milk. International Dairy Journal, **16**: 555–562.

Deeth H.C., Fitz-Gerald C.H. (1995): Lipolytic enzymes and hydrolytic rancidity in milk and dairy products. In: Fox P.F. (ed.): Advanced Dairy Chemistry. Vol. 2. Lipids. 2nd Ed. Chapman & Hall Publishers, London: 247–308.

Ducháček J., Vacek M., Stádník L., Beran J., Okrouhlá M. (2012): Changes in milk fatty acid composition in relation to indicators of energy balance in Holstein cows. Acta Universitatis Agriculturae et Silviculturae Mendeleianae Brunensis, **60:** 29–38.

Evers J.M. (2004): The milkfat globule membrans – Methodologies for measuring milkfat globule (membrans) damage. International Dairy Journal, **14**: 747–760.

Evers J.M., Palfteyman K.R. (2001): Free fatty acid levels in New Zealand raw milk. Australian Journal of Dairy Technology, **56**: 198–201.

Forman L. (1984): Lipolýza mléčného tuku (I. část). Mlékařské listy: 310–313

Frelich J., Šlachta M., Hanuš O., Špička J., Samková E. (2009): Fatty acid composition of cow milk fat produced on low-input mountain farms. Czech Journal of Animal Science, **54**: 532–539.

González-Martín I., Hernández-Hierro J.M., Revilla I., Vivar-Quintana A., Morón-Sancho R., Salvador-Esteban J. (2009): A comparative study of the analysis of free fatty acids in cheeses (cow's, ewe's and goat's) with different ripening times using nirs: intact samples and fat extracts. Czech Journal of Food Sciences, **27** (Special Issue 1): S114–D118.

González F.D., Muiño R., Pereira V., Campos R., Benedito J.L. (2011): Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. Journal of Veterinary Science, **12**: 251–255.

Hanuš O., Janů L., Vyletělová M., Macek A., Zajíčková I., Kopecký J., Jedelská R., Nejeschlebová L. (2004): Vliv faktorů prvovýroby jako genotypu dojnice, krmení a bakteriální a mykotoxinové kontaminace mléka na jeho technologické ukazatele typu obsahu volných mastných kyselin, kysací schopnosti a syřitelnosti. In: Sborník příspěvků Aktuální problémy řízení v chovu skotu, Rapotín: 32–54.

Hanuš O., Vegricht J., Frelich J., Macek A., Bjelka M., Louda F., Janů L. (2008): Analysis of raw cow milk quality according to free fatty acid contents in Czech Republic. Czech Journal of Animal Science, **53**: 17–30.

Hanuš O., Frelich J., Tomáška M., Vyletělová M., Genčurová V., Kučera J., Třináctý J. (2010): The analysis of relationships between chemical composition, physical, technological and health indicators and freezing point in raw cow milk. Czech Journal of Animal Science, **55**: 11–29.

Herdt T. (2007): Postabsorptive nutriet utilization. In: Cunningham J.G., Klein B.G. (eds): Veterinary Physiology. 4th Ed. Saunders/Elsevier, St. Louis: 389–407.

IDF (1987): Significance of lipolysis in the manufacture and storage of dairy products. IDF Bulletin, No. 265. International Dairy Federation, Brussel.

Kalač P., Samková E. (2010): The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. Czech Journal of Animal Science, **55**: 521–537.

Kon H., Saito Z. (1997): Factors causing temperature activation of lipolysis in cow s milk. Milchwissenschaft, **52**: 435–440.

Kulig H., Kowalewska-Łuczak I., Kmieć M., Wojdak-Maksymiec K. (2010): ANXA9, SLC27A3, FABP3 and FABP4 single nucleotide polymorphisms in relation to milk production traits in Jersey cows. Czech Journal of Animal Science, **55**: 463–467.

Miller B., Puhan Z. (1985): Das „lipolysierbare"Fett als Indikator für die Fettschädigung in der Rohmilk. Schweiz Milchwissenschaft Forschung, **15**: 10–17.

Navrátilová P., Borkovcová I., Vyhnálková J., Vorlové L. (2011): Fluoroquinolone residues in raw cow's milk. Czech Journal of Food Sciences, **29**: 641–646.

Nelson D.L., Cox M.M. (2000): Integration and hormonal regulativ of mammalian metabolism. In: Nelson D.L., Cox M.M. (eds): Lehninger Principles of Biochemistry. 3rd Ed. Worth Publishers, New York: 869–890.

Poláková K., Kudrna V., Kodeš A., Hučko B., Mudřík Z. (2010): Non-structural carbohydrates in the nutrition of high-yielding dairy cows during a transition period. Czech Journal of Animal Science, **55**: 468–478.

Quattar G.C., Jeon I.J., Hart-Thakur R.A., Schmidt K.A. (2004): Fatty acids released from milk fat by lipoprotein lipase and lipolytic psychotrophs. Journal of Food Science C: Food Chemistry and Toxicology, **68**: 659–664.

Samková E., Pešek M., Špička J., Pelikánová T., Hanuš O. (2009): The effect of feeding diets markety differing in the proportion of grass and maize silages on bovine milk fat composition. Czech Journal of Animal Science, **54**: 93–100.

SAS (2004): SAS/STAT® 9.1. User's Guide. SAS Institute Inc., Cary.

Sojková K., Hanuš O., Říha J., Genčurová V., Hulová I., Jedelská R., Kopecký J. (2010): Impacts of lactation physiology at higher and average yield on composition, properties and health indicators of milk in holstein breed. Scientia Agriculturae Bohemica, **41**: 21–28.

Wilking L., Nielsen J.H., Bavius A.K., Edvardsson A., Svennersten-Sjaunja K. (2006): Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. Journal of Dairy Science, **89**: 1004–1009.

Received for publication January 12, 2012
Accepted after corrections March 29, 2012

Corresponding author

Ing. Renáta Toušová, CSc., Česká zemědělská univerzita, Fakulta agrobiologie, potravinových a přírodních zdrojů, Kateda speciální zootechniky, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika; E-mail: tousova@af.czu.cz