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MILK FAT FREE FATTY ACIDS IN DEPENDENCE ON HEALTH OF DAIRY COWS

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Abstract

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Free fatty acids (FFAs) in fat are important indicator of raw milk quality. The aim was to analyse the relationships and quantify effects of dairy cow mastitis on FFAs. Individual milk samples (IMSs; n 307) came form Holstein and Czech Fleckvieh cows (67 : 33%). Sampling was from 10 to 270 days in milk for 1 year. Cows were fed in stables (n 5) by total mixed ration. Herd lactation milk yield varied from 7000 to 10 000 kg. Milking was performed twice a day using pipeline milking equipment and in milking parlours (20 : 80%). IMSs were classified into 3 groups according to somatic cell count (SCC): A ≤ 150, healthy; B from 800 to 1500, subclinical mastitis; C > 1500 10^3.ml^{-1}, subclinical and clinical mastitis. Lactose (L) decreased with growing intensity of mammary gland secretion disorder from 4.90 ± 0.278 (A) to 4.49 ± 0.44% (C; P < 0.001). The values increased in the same direction: content of FFAs from 0.65 ± 0.209 to 1.44 ± 0.379 mmol.$100g^{-1}$ (P < 0.001); milk energy quotients fat/protein (F/P) from 0.88 ± 0.386 to 1.23 ± 0.335 (P < 0.001) and fat/lactose (F/L) from 0.63 ± 0.266 to 1.98 ± 0.309 (P < 0.001). The mastitis state (SCC) deteriorated milk quality by increase of FFAs, 0.703 (P < 0.001; by 121.5%) and reduced L, –0.522 (P < 0.001). 49.4% of FFA variability could be explained by SCC variations. Increased SCC by 1000 10^3.ml^{-1} increased FFAs by 0.433 mmol.$100g^{-1}$. The L decrease due to worse mastitis state or cow energy malnutrition increased FFAs, –0.312 (P < 0.001). A risk increase of ketosis coefficients (F/P and F/L) deteriorated milk quality by increase of FFAs, 0.395 and 0.443 (P < 0.001) where 0.1 is equal to 0.05 and 0.067 mmol.100 g^{-1}. Values of FFAs can serve to control the health of dairy cows and quality of IMSs in regular milk recording.

Key words: raw cow milk quality; mastitis; somatic cell count; lactose; ketosis quotient
Abbreviations: C – casein; CA – citric acid; CSN – Czech standard; F – fat; FFAs – free fatty acids; IMSs – individual milk samples; IR – infrared; L – lactose monohydrate; MFP – milk freezing point depression; MI – milk indicator; MIR–FT – infrared spectrometry with Fourier´s transformation; P – crude protein; PT – proficiency testing; SCC – somatic cell count; SNF – solids non fat; U – urea

Introduction

Current content of milk fat free fatty acids (FFAs) lies between 0.5 and 1.2 and maximal enabled is 1.3 for method by churning and 3.2 mmol.100 g^{-1} for method by extraction and titration according to CSN 57 0529 and CSN 57 0533. Increase of FFAs means row of negative impacts like metabolic problems of cows and enzymatic lipolysis (Figure 1). High FFAs cause an aggravation of milk technological properties (Vyletělová et al., 1999, 2000 a, b) and deterioration of

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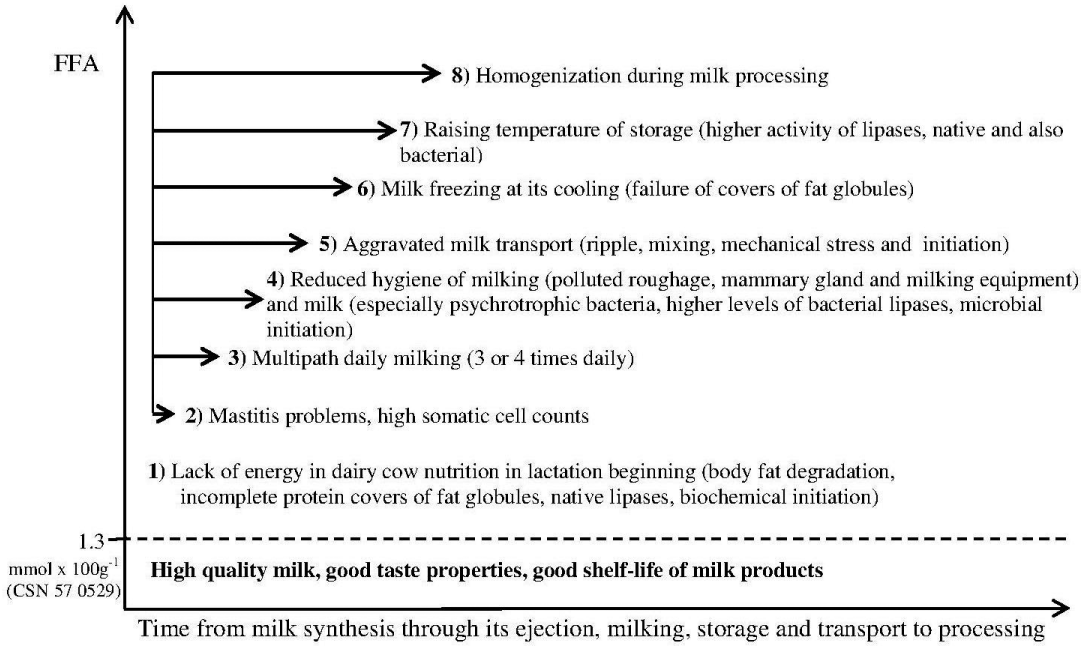

Fig. 1. Lipolysis rise of fat in raw milk, content increase of free fatty acids (FFA), threat of milk and dairy product quality – factors and their combinations, related to animal and technology (modified according to Sjaunja (1984), O´Brian et al. (1998), Vyletělová et al. (2000 a), Antonelli et al. (2002), Hanuš et al. (2004, 2008), Thomson et al. (2005), Deeth (2006); Wiking et al. (2006) and Mikulová (2011)

sensory milk properties. Therefore, it is important property for milk processing. Lipases can be thermoresistent and thus in this way to influence milk also after its heat treatment by dairy product degradation.

Wasteful milk handling as often pumping and ripple at manipulation (Sjaunja, 1984; Thomson et al., 2005; Hanuš et al., 2008) and its freezing on technology surfaces can induce a lipolysis. Poor hygiene of dairy cow stabling and milking as well as bad storage and treatment of raw milk can lead to propagation of undesirable psychrotrophic, thermoresistant and sporulating milk microflora (Vyletělová et al., 1999; Cempírková, 2002, 2007; Dankow et al., 2004; Hanuš et al., 2004; Deeth, 2006; Foltys and Kirchnerová, 2006; Hantis–Zacharov and Halpern, 2007; Cempírková and Mikulová, 2009; Toušová et al., 2013). The mentioned facts can increase the lipolysis intensity (Figure 1).

Thus, results of FFAs are important at raw milk quality assessment for its processing on products. Despite of this fact these analyses are not carried out very often in bulk milk samples because of labour demandingness of original reference method in practice. Currently, after the start of MIR–FT (infrared spectrometry with Fourier´s transformation) (Bijgaart van den, 2006; Hanuš et al., 2008, 2013 b) routine indirect analytical method, there is a new possibility to analyse larger sets of IMSs as well. Thus, it will be possible to use the evaluated relationships between indicators of IMSs for advisory service to good milk quality and cow health at milk recording analyses.

The goal of this paper was to analyse and explain the basic relationships and quantify possible effects of dairy cow mastitis state and milk secretion disorders respectively on concentration of raw milk fat free fatty acids directly and indirectly in this way on quality and shelf life of milk products and milk food chain safety.

Materials and Methods

Animal conditions and individual milk samples

Individual milk samples (IMSs) were collected during whole year over all seasons (n = 307). These came form dairy cows of Holstein and Czech Fleckvieh breed in north Moravia in ratio 67 : 33%. Cows were sampled from early lactation to 270 days in milk. Daily milk yield of these animals varied from 10 to 34 kg according to lactation stage and curve. All animals were kept in stables for whole year without pasture and fed by total mixed ration (roughage as maize, alfalfa, clover and clover-grass silage and hay (specifically in dependence on herd, n = 5 with milk yield from 7000 to 10 000 kg per standard lactation) with supplements

of concentrates, mineral mixtures and vitamins according to current milk yield). Animals were kept in binding and free stables which were in ratio 20 : 80%. Dairy cows were fed and milked twice a day and milking was performed using pipeline milking equipment and in milking parlours (also 20 : 80%). The sampling was carried out using flow milkmeters (Tru-Test, New Zealand) and also dripping bottles in some milking parlours. IMSs were selected for experimental investigation and purposes using extremely marked somatic cell count ((SCC) ≤ 150 as healthy and ≥ 800 10^3.ml^{-1} as suspect) and fat ((F) > 1 and < 7%) value because of possible highlighting of studied relationships (SCC classification) and avoidance of possible interference effects (F limitation). The samples were transported under refrigerator conditions (< 8°C) in native state without preservation to laboratory and immediately analysed as follows.

Used milk indicators and relevant analytical methods

Infrared (IR) milk analyzer Lactoscope FTIR (Delta Instruments, The Netherlands) working in mid range of the IR spectrum with Fourier´s transformation (MIR–FT) was used for determination of milk indicators (MI) such as F (fat), P (crude protein), C (casein), L (lactose monohydrate), SNF (solids non fat, all previous contents in %), U (urea concentration in mg.100 ml^{-1}), CA (citric acid concentration in mmol.l^{-1}), MFP (milk freezing point depression in °C) and FFAs (milk fat content of free fatty acids in mmol.100 g^{-1}). Milk SCC (somatic cell count in 10^3.ml^{-1}) was investigated by the flow cytometry using Somacount FC (Bentley Instrument, USA). Both instruments were calibrated for various measurements of MI according to the results of relevant reference methods regularly with acceptable results. These instruments took part in the official proficiency testing (PT) of result reliability with good level. The MIR–FT measurement of FFAs was calibrated (Figure 2) (IDF 1991; Foss, 2001, 2004; Bijgaart van den, 2006) according to results of so called churning method (Koops et al., 1990; by titration using KOH solution) of determination of FFAs (CSN 57 0533 and 57 0529) with use of specific adjustment procedure (Hanuš et al., 2008, 2013b). This instrument also took part in relevant PT on national level regularly with satisfactory evaluation.

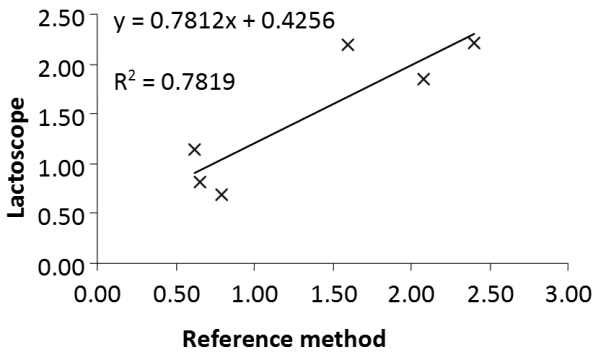

Fig. 2. The result of Lactoscope (MIR–FT) calibration on measurement of FFAs (mmol.100g^{-1}) according to reference method results (n = 6; correlation coefficient 0.884, P < 0.001)

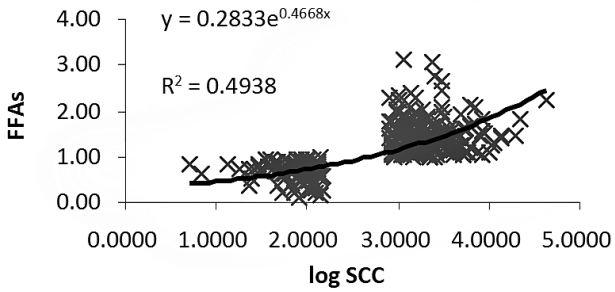

Fig. 3. The relationship between somatic cell count (log SCC) and free fatty acids (FFAs; mmol.100g^{-1}) in chosen individual cow milk samples (n = 307; correlation index 0.703, P < 0.001)

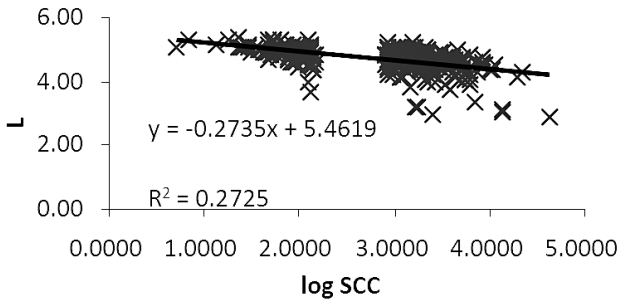

Fig. 4. The relationship between somatic cell count (log SCC) and lactose (L; %) in chosen individual cow milk samples (n = 307; correlation coefficient –0.522, P < 0.001)

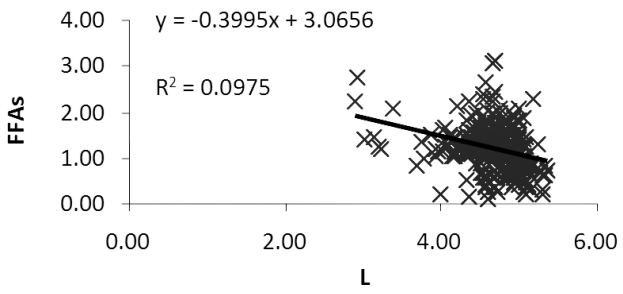

Fig. 5. The relationship between lactose (L; %) and free fatty acids (FFAs; mmol.100g^{-1}) in chosen individual cow milk samples (n = 307; correlation coefficient –0.312, P < 0.001)

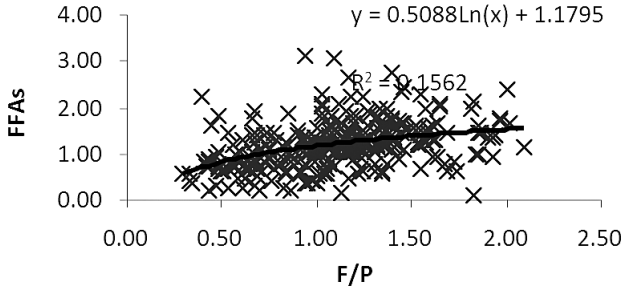

Fig. 6. The relationship between fat/protein ratio (F/P) and free fatty acids (FFAs; mmol.100g^{-1}) in chosen individual cow milk samples (n = 307; correlation index 0.395, P < 0.001)

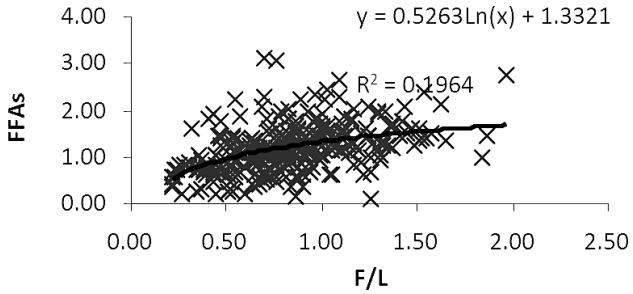

Fig. 7. The relationship between fat/lactose ratio (F/L) and free fatty acids (FFAs; mmol.100g–1) in chosen individual cow milk samples (n = 307; correlation index 0.443, P < 0.001)

SCC and FFAs data classification

Individual milk samples were classified and thus data about FFAs and other milk indicators were distributed into three groups according to SCC (as main indicator of milk secretion disorders and mammary gland health, in accordance) (Reneau et al., 1986; Pytlewski et al., 2012) values: A ≤ 150 10^3.ml^{-1} as healthy mammary gland (n = 94); B from 800 to 1500 10^3.ml^{-1} as problem with clear subclinical mastitis occurrence which can contribute to bulk milk as dairy plant delivery under practice conditions, it can influence of milk product quality (n = 86); C > 1500 10^3.ml^{-1} as trouble with subclinical and clinical mastitis occurrence and such milk is usually cancelled of dairy plant delivery and thus it does not affect milk product quality (n = 127).

Statistical procedures

Evaluation of FFAs MIR–FT calibration results was performed by linear regression in accordance with relevant system of statistical treatment (Figure 2). The main statistical parameters as means, standard deviations and variation coefficients were calculated (Table 1 and 2) for milk sample data sets and mean differences between SCC groups (A, B and C) of milk samples were tested by t–test (Table 3). Milk energy or ketosis quotients were calculated for milk sample results as ratio F/P and F/L. Logarithmic ($\log_{10}$) transformation of SCC data was performed because of absence of normal frequency distribution of these values (Reneau et al., 1986; Hanuš et al., 2008; Yilmaz and Koc, 2013) especially in individual milk samples where arithmetic mean is not suitable parameter for sets of SCC data and therefore in this way also geometric

Table 1
Main statistic characteristics of indicators of individual milk samples (n = 307)

	x	xg	sd	v	min	max	m
SCC	2068		3577	173	5	43181	1221
log SCC	2.8666	736	0.744		0.699	4.6353	3.0867
F	3.85		1.298	33.7	1.03	6.97	3.81
P	3.51		0.431	12.3	2.58	4.7	3.45
L	4.68		0.39	8.3	2.88	5.35	4.73
SNF	8.81		0.478	5.4	7.56	10.13	8.81
C	2.79		0.369	13.2	1.93	3.93	2.73
U	38.37		9.734	25.4	7.11	68.77	39.12
CA	8.092		0.28	3.5	7.68	11.17	8.05
FFAs	1.2		0.499	41.7	0.12	3.14	1.18
F/P	1.11		0.38	34.3	0.29	2.09	1.12
F/L	0.84		0.322	38.4	0.21	1.97	0.82

x – arithmetic mean; xg – geometric mean; sd – standard deviation; v – variation coefficient (%); min – minimum; max – maximum; m – median; SCC – somatic cell count, 10^3.ml^{-1}; F – fat, %; P – crude protein, %; L – lactose monohydrate, %; STP – solids non fat, %; C – casein, %; U – urea, mg.100 ml^{-1}; CA – citric acid, mmol.l^{-1}; FFAs – free fatty acids, mmol.100g^{-1}; F/P – ratio fat/crude protein; F/L – ratio fat/lactose

means were used. Mutual relationships between milk indicators were evaluated by linear and nonlinear regression as well (Figure 3–7). The MS Excel (Microsoft, Redmond, USA) programme was used for this statistic evaluation.

Results and Discussion

The basic statistic results of data set (Table 1) about effect of SCC (sample groups A, B and C) on chosen milk

Table 2

Main statistic characteristics of indicators of individual milk samples according to SCC groups

		n	x	xg	sd	v	min	max	m
A	SCC	94	82		37	45,1	5	149	82
	log SCC	94	1.8479	70	0.278		0.699	2.1732	1.9111
	F	94	3.05		1.256	41.2	1.03	6.25	2.82
	P	94	3.53		0.473	13.4	2.58	4.49	3.46
	L	94	4.9		0.278	5.7	3.69	5.35	4.94
	SNF	94	9.05		0.491	5.4	8.03	10.13	9
	C	94	2.8		0.412	14.7	1.93	3.64	2.74
	U	94	38.33		9.326	24.3	13.49	61.43	39.36
	CA	94	8.157		0.175	2.2	7.82	8.79	8.13
	FFAs	94	0.65		0.209	32.2	0.12	0.99	0.66
	F/P	94	0.88		0.386	44.1	0.29	1.93	0.81
	F/L	94	0.63		0.266	42.4	0.21	1.3	0.6
B	SCC	86	1112		206	18.5	808	1478	1109
	log SCC	86	3.0389	1094	0.081		2.9074	3.1697	3.0449
	F	86	4.03		1.131	28.1	1.44	6.97	3.88
	P	86	3.42		0.397	11.6	2.63	4.7	3.36
	L	86	4.7		0.261	5.5	3.78	5.25	4.75
	SNF	86	8.74		0.407	4.7	7.67	10.03	8.72
	C	86	2.72		0.336	12.3	2.05	3.93	2.68
	U	86	37.61		10.853	28.9	7.11	67.09	38.81
	CA	86	8.021		0.118	1.5	7.8	8.39	8.01
	FFAs	86	1.44		0.399	27.7	1	3.14	1.32
	F/P	86	1.18		0.322	27.2	0.45	2.09	1.18
	F/L	86	0.86		0.274	31.7	0.32	1.84	0.82
C	SCC	127	4186		4792	114.5	1508	43 181	2737
	log SCC	127	3.504	3192	0.279		3.1784	4.6353	3.4373
	F	127	4.32		1.159	26.8	1.58	6.82	4.26
	P	127	3.55		0.416	11.7	2.86	4.61	3.5
	L	127	4.49		0.44	9.8	2.88	5.19	4.6
	SNF	127	8.69		0.452	5.2	7.56	9.75	8.67
	C	127	2.83		0.352	12.5	2.24	3.77	2.77
	U	127	38.91		9.261	23.8	12.65	68.77	38.93
	CA	127	8.093		0.39	4.8	7.68	11.17	8.04
	FFAs	127	1.44		0.379	26.4	1	3.07	1.35
	F/P	127	1.23		0.335	27.3	0.39	2.01	1.19
	F/L	127	0.98		0.309	31.6	0.35	1.97	0.92

n – number of cases; A, SCC ≤ 150 $10^3.ml^{-1}$, healthy mammary gland; B, SCC from 800 to 1500 $10^3.ml^{-1}$, marked subclinical mastitis; C, SCC > 1500 $10^3.ml^{-1}$, subclinical and clinical mastitis occurrence

Table 3
Significance test of mean differences between SCC groups of individual milk samples

	A – B		A – C		B – C	
	t	sig	t	sig	t	sig
SCC	47.5	***	8.26	***	5.92	***
log SCC	38.03	***	43.51	***	14.99	***
F	5.44	***	7.73	***	1.81	ns
P	1.68	ns	0.38	ns	2.33	*
L	4.89	***	7.84	***	3.93	***
SNF	4.49	***	5.61	***	0.9	ns
C	1.44	ns	0.43	ns	2.14	*
U	0.48	ns	0.46	ns	0.94	ns
CA	6.01	***	1.46	ns	1.67	ns
FFAs	16.7	***	18.06	***	0.07	ns
F/P	5.74	***	7.14	***	0.9	ns
F/L	5.87	***	8.78	***	2.7	**

A – B, A – C and B – C – differences between SCC groups; t – t–test criterion; sig – difference significance; ns – insignificant, $P > 0.05$; * – significant, $P \leq 0.05$; ** – $P \leq 0.01$; *** – $P \leq 0.001$

indicators and especially on FFAs are shown in Table 2. In A group (healthy dairy cows), the largest variability of milk indicators was, in exception of SCC, in FFAs, fat, F/L and F/P (from 32.2 to 44.1%) and the lowest than in CA, SNF and L (from 2.2 to 5.7%) (Table 2). Statistic significance of mutual differences between milk indicator means of A, B and C group is shown in Tab. 3. Geometric means of SCC for A, B and C were 70, 1094 and 3192 $10^3.ml^{-1}$ (Table 2) and these were mutually different ($P < 0.001$; Table 3). This significance was logical in dependence on selection of IMSs. The significant differences (Table 3) at least between two groups were noted for most of observed milk indicators in dependence on mentioned distribution: L; SNF; casein; CA; FFAs; F/P; F/L. Only milk urea was not affected along groups due to carried out selection of samples (Table 2 and 3). Protein, casein and milk citric acid were influenced by mastitis state of dairy cows in less marked way.

Marked effects with clear trend caused by health SCC groups were noted in fat, SNF and especially in L, FFAs and milk ketosis quotients F/P and F/L (Table 2 and 3). Lactose decreased logically with growing intensity of mammary gland secretion disorder from 4.90 ± 0.278 (A) to 4.49 ± 0.44% (C; $P < 0.001$; Table 2 and 3). The following values increased also in the same direction with worsening health: content of FFAs from 0.65 ± 0.209 to 1.44 ± 0.399 (B) and 1.44 ± 0.379 mmol.100 g^{-1} ($P < 0.001$); milk energy quotients F/P from 0.88 ± 0.386 to 1.23 ± 0.335 ($P < 0.001$) and F/L from 0.63 ± 0.266 to 1.98 ± 0.309 ($P < 0.001$).

The chosen relationships between investigated indicators with respect to mammary gland health state and cow energy metabolism milk indicators are shown in charts from Figures 3–7. The mastitis state (SCC) deteriorated milk quality markedly in terms of significant increase of FFAs with correlation index 0.703 ($P < 0.001$; Figure 3) and also reduced L with correlation coefficient –0.522 ($P < 0.001$; Figure 4) logically. The L decrease due to worsened mastitis state or possible cow energy malnutrition increased the FFAs, –0.312 ($P < 0.001$; Figure 5). On the contrary the risk increase both milk ketosis quotients (F/P and F/L) deteriorated milk quality by marked increase of FFAs with correlation indexes 0.395 and 0.443 (both $P < 0.001$) (Figure 6 and 7) similarly.

About possible mastitis (SCC) effect on content of FFAs was speculated already previously according to some papers (Figure 1) (Sjaunja, 1984; O'Brian et al., 1998; Vyletělová et al., 2000a; Antonelli et al., 2002; Hanuš et al., 2004, 2008; Thomson et al., 2005; Wiking et al., 2006; Mikulová, 2011). However, the direct dependence with factor quantification has not been mentioned. Here, the experiment about quantification of this effect (Table 2) showed increase of FFAs by 121.5% as consequence of effects of subclinical (B) or subclinical and clinical mastitis (C). Technologically, it could be a part of spontaneous lipolysis (Toušová et al., 2013) but from physiological point of view this is induced lipolysis. 49,4% of FFA variability (Figure 3) could be explained by SCC variations. Increased SCC (due to mastitis) by 1000 $10^3.ml^{-1}$ increased FFAs by 0.433 mmol.100 g^{-1} (calculated from linear regression). Thomson et al. (2005) did not shown so close relationships in bulk milk samples although their relationships had the same trend in comparison to our findings. The carried out animal classification into groups (A,

B and C) with ambition to emphasize the effect of SCC on differences in milk quality (FFA) could be one of the reasons why here obtained closeness of relationships – Figure 3 (for SCC) and 5 (for L); 0.703 and –0.312, $P < 0.001$) was markedly higher than in previous papers (for SCC 0.07 and for L –0.14, $P < 0.05$ and $P < 0.01$) (Hanuš et al., 2008 and Thomson et al., 2005). The second reason is probably experiment with IMSs whereas other papers mentioned mostly bulk milk samples (Thomson et al., 2005; Hanuš et al., 2008; Mikulová, 2011). In these the impact can be soften down due to a diluting effect of healthy milk, it means without stress caused by subclinical production disorder occurrence. Lactose content which was reduced by secretion disorders (Figure 5) can explain 9.8% of variability in the occurrence of FFAs. With lactose decrease by 0.1% the FFA content grew up by 0.04 mmol.100 g^{-1}.

As it has been defined (Geishauser and Ziebell, 1995; Steen et al., 1996; Geishauser et al., 1997; Duffield et al., 1997; Heuer et al., 2000; Siebert and Pallauf, 2010; van Knegsel et al., 2010; Hanuš et al., 2011, 2013a, c; van der Drift et al., 2012; Manzenreiter et al., 2013), milk F/P ratio is suitable indicator of dairy cow energy balance and its high value (for instance > 1.3) is indicator of suspicion from subclinical ketosis occurrence. Correlation between F/P and FFAs are not known for comparison purposes. Here, 39.5% of FFA variability (Figure 6) is explainable by variations in F/P ratio. Also milk F/L ratio is good indicator of dairy cow energy balance (Steen et al., 1996; Reist et al., 2002; Hanuš et al., 2013a, c; Manzenreiter et al., 2013). Also its higher value (for example > 0.9) is indicator of suspicion from subclinical ketosis occurrence. Correlation between F/L ratio and FFAs are not known for comparison purposes as well. Here, 19.6% of variability in FFAs (Figure 7) is explainable by variations in values of F/L. Therefore, the increase of F/P and F/L values (in linear interpretation) by 0.1 in consequence of nutritional energy problems of dairy cows is equal to the growth of FFAs by 0.05 and 0.067 mmol.100g^{-1}.

Conclusion

Significant relationships of SCC and both milk ketosis quotients (F/P and F/L) to FFAs show on diagnostical ability of FFA content not only regarding milk secretion disorders but also dairy cow energy troubles. Values of FFAs in IMSs (in regular milk recording) can serve to control the health of dairy cows in terms of suspicion from secretion or energy balance disorder occurrence or raw milk quality in consideration of quality and shelf-life of resulting milk products.

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