Analysis of raw cow milk quality according to free fatty acid contents in the Czech Republic

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ABSTRACT: The concentration (*c*) of free fatty acids (FFAs) in milk is an indicator of dairy cow nutrition, milk straining, its bacterial contamination and storage quality. High FFA concentrations (*cs*) caused by lipolysis can damage the quality properties of milk products. Therefore the FFA content is introduced thanks to an increase in the efficiency of modern analytical methods as a milk quality indicator and as an indicator for its price as well. The goal of this paper was to analyse the FFA relations to the other milk quality indicators. The data set (n = 11 586) was evaluated by regression methods. In November and December the respective FFA means were 0.614 ± 0.458 and 0.835 ± 0.491 mmol/100 g with a relatively high variability of 74.6 and 58.8%. The frequency of unsatisfactory FFA values (> 1.3) was 7.51 and 13.93%. Casein content (r = –0.17; P < 0.01) and crude protein content (r = –0.12; P < 0.01) were related more closely with FFA *c*. The FFAs can increase by 0.066 mmol/100 g with casein decrease by 0.10%. The FFAs in milk fat can slightly increase by the supply of energy to dairy cows (protein and casein decrease) and rise with the deteriorating health state of mammary gland (lactose, r = –0.14; P < 0.01) as well. The somatic cell count correlated with FFAs more weakly (r = 0.07; P < 0.05), similarly like the total mesophilic bacteria count (r = 0.11; P < 0.01), relatively more closely the psychrotrophic bacteria count (r = 0.27; P < 0.05). The deterioration of almost all hygienic indicators signified an FFA *c* increase. The urea content correlated with FFAs weakly (r = –0.08; P < 0.05) and the fat content imperceptibly as a component of similar substance like FFAs. The mechanical milk stress led to FFA liberation from fat esters proportionally to the intervention intensity (P < 0.001). Even a relatively small mechanical stress caused by mixing comparable to the current milking technology, milk transport and storage increased the FFA *c* of milk fat from 1.11 ± 0.19 to 1.80 ± 0.40 mmol/100 g (P < 0.05). The highest experimental stress up to 6.88 ± 0.55 mmol/100 g (P < 0.001).

Keywords: cow; raw milk; bulk milk sample; mammary gland; fat; free fatty acids; crude protein; casein; lactose; somatic cell count; total mesophilic bacteria count; psychrotrophic bacteria count; urea; mechanical stress; lipolysis

Milk fat precursors in mammal blood are first of all volatile fatty acids (VFA, acetic, propionic and butyric acid) from the process of starch and cellulose rumen fermentation. Further fats from fodder (Komprda et al., 2005; Pešek et al., 2006; Strusińska et al., 2006) or organism fat reserves with energy state changes (Baer, 1991), ketose, liver steatose or lipomobilization syndrome and/or glucose are dis-

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cussed, also the possible effect of rumen-protected protein supplemented with three amino acids (Třináctý et al., 2006). A small portion of fatty acids not esterified in triglycerides is freely dispersed chiefly in the milk fat phase and slightly in the milk water phase and is termed free fatty acids (FFAs). The usual FFA content in milk fat is between 0.5 and 1.2 mmol/100 g (the maximum allowed is 13.0 mmol/kg for a churning method or 32.0 mmol per kg for an extraction method; ČSN 57 0529, 1993). Gerber's acidobutyrometric method captures up to 90% of FFA content into the milk fat portion, on the contrary Roese-Gottlieb's extraction-gravimetric method does not include FFAs into the fat portion so reliably losing up to 70% of them (Kerkhoff Mogot et al., 1982). Therefore possible lipolysis in milk samples causes certain differences between the fat content captured by means of these two methods. The higher the lipolysis grade, the higher the mentioned differences for identical samples.

The FFA content increase implies negative impacts of lipolysis type or defective fat globule generation, usually for the reason of dairy cow metabolic problems. An increased FFA *c* causes deterioration of technological milk properties (Vyletělová et al., 2000a,b), but mainly deterioration of milk sensory properties, taste and odour. The consequence is a slightly bitter smack which can impair the dairy product quality. The increased FFA content also reduces or even inhibits the milk fermentation progress in soured product production (Peterková, 2002). Some fatty acids possess evident bacteriostatic or even bactericidal effects, which is naturally also used in the mammary gland defence system in the form of a teat duct keratin plug with free fatty acid content. It is a case of udder antimastitis immunity. The above stated is a reason why FFA contents in milk are currently understood and used as a milk quality indicator with a possible influence on its evaluation. An increased FFA content points either to the deteriorated health state of dairy cow or to fat decomposition as a consequence of milk microbial contamination which is usually caused by undesirable psychrotrophic or thermoresistant microflora (Shelley et al., 1987; Vyletělová et al., 1999a,b, 2001; Hanuš et al., 2004, 2005). It can be the consequence of insufficient milk storage or its excessive mechanical straining, e.g. drawing just as during its further technological treatment.

The fat destruction is then a phenomenon caused in milk by natural enzymes (lipases) or lipases supplied by milk bacterial contamination. Lipolysis is therefore spontaneous or induced. At the same time lipases can be thermoresistant and show themselves so even after a milk heat treatment by decomposition of dairy products (Peterková, 2002). Due to a number of changes in primary production like disinfection and cooling storage the portion of psychrotrophic and thermoresistant microflora grows, which is a significant lipase and protease carrier (Vyletělová et al., 1999a,b, 2000a,b, 2001; Hanuš et al., 2004, 2005) and thus a factor of possible milk putrefaction. The FFA monitoring in milk is thus important for both the dairy cow health and the quality and safety of dairy food chain as well as for the health of dairy product consumers.

The incomplete formation of fat globule protein coatings as a consequence of metabolic problems (dairy cow energy malnutrition – negative balance at the beginning of lactation) and mechanical damage to fat globule coatings make the fat molecules accessible to the enzyme action and facilitate the fat decomposition. The nutrient influences on FFA contents in milk are contained especially in their seasonal trends. Roubal et al. (2006) reported the highest values for raw cow milk in the Czech Republic in September and October (0.98 and 0.97 mmol/100 g) and the lowest in April and May (0.72 and 0.71 mmol/100 g) with a year average of 0.82 mmol/100 g of fat. The seasonal effect on FFA content in milk fat was significant ($P < 0.05$).

An inconsiderate milk treatment such as frequent drawing and clarification during manipulation and icing also creates their own lipolysis. The supplied thermal or mechanical energy into the multicomponent milk system damages the fat globule membranes and liberates fatty acids from the triglyceride ester link. Therefore the milk flow should not exceed a velocity of 1 up to 1.5 m/s. In such a case the laminar flow can turn into a mechanically more aggressive turbulent one (Peterková, 2002). Higher velocities, especially over 2.7 m/s, increase the fat content. It occurs primarily in the piping reduction.

Insufficient cow housing and milking hygiene just as bad raw milk storage and treatment can lead to the initiation of undesirable psychrotrophic, thermoresistant and sporulating milk microflora. It can increase lipolysis intensity. In the past this problem was also investigated at our workplace (Vyletělová and Hanuš, 2000; Hanuš et al., 2004).

The negative energy balance of cows in view of their increasing milk yield can lead to lipomo-

bilisation and ketose syndrome initiation. These health and production disorders are initiated by uncontrolled katabolism connected with body fat liberation for energy acquirement. Thus at first the fat and FFA contents increase in blood and milk as well. There are also other factors increasing the FFA content in milk such as mastitis disorder with an increased somatic cell count in milk, late lactation stage, and also shorter intervals between milkings. That can initiate even multinumerous daily milking. Another factor is the bad quality of particularly bulky fodders such as silage and hay. If it comes to a rapid and repeated apparent FFA content increase in bulk milk samples on the farm, e.g. after reconstruction of old milking machines or after installation of new ones, the defect can be looked for in the technical equipment function. If nothing important was carried out with machine milking and in spite of that an undesirable FFA content increase occurred as against the preceding conditions, the defect can be caused by inadequate milk storage; worse milking hygiene; deteriorated fodder quality; unbalanced nutrition and deteriorated cow health state in terms of increased occurrence of production disorders of mastitis type, negative energy balance, liver steatose or ketose.

MATERIAL AND METHODS

Evaluated sets of bulk milk samples

The bulk milk samples of large data sets (I and II) were regularly obtained (once or several times per month) from commercial dairy herds for the milk quality determination (mostly according to the standard ČSN 57 0529, 1993) in the framework of the official milk payment system during two calendar months in 2003 (November (NOV) and December (DEC)). The samples were treated with a low temperature of about 6°C and immediately transported to the accredited milk laboratory. Some of them were preserved, some of them not, but the milk samples were generally analysed in accordance with the relevant standard operation procedures of the accredited laboratory. The milk samples came from both the milked populations of dairy cows in this country, Holstein cattle and Bohemian Pied cattle. Different numbers of milk samples were investigated for different indicators. The maximum sample numbers were for FFA (n = 5 840 and 5 746).

In a small trial, five bulk milk samples (0.5 l each; P) were exposed to mechanical stress by stirring in six steps (from I to VI) according to a description in Table 5. The FFAs cs were measured in all the samples subsequently.

Investigated MQIs with their abbreviations and units

With tested milk quality indicators (MQIs) that were measured and calculated the following listed abbreviations and units were used:

F = milk fat content (g/100 ml; %);
L = lactose content (monohydrate; g/100 g; %);
SNF = solids-not-fat content (g/100 g; %);
DM = dry matter (g/100 g; %; calculated indicator);
CP = crude protein (total N × 6.38; g/100 g; %);
CAS = casein (casein N × 6.38; g/100 g; %);
WP = whey protein content (g/100 g; %; calculated indicator);
MFP = milk freezing point (°C or m°C × (–1));
SCC = somatic cell count (ths × ml^{-1});
F/CP = fat to crude protein ratio, calculated indicator of nitrogen/protein metabolism of dairy cow herd;
U = urea c (mmol/l);
FFA = c of milk fat free fatty acids (mmol/100 g);
TMBC = total mesophilic bacteria count (ths. CFU × ml^{-1});
CBC = coli bacteria count (CFU/ml);
TRBC = thermoresistant bacteria count (CFU/ml);
PBC = psychrotrophic bacteria count (CFU per ml)

Analytical methods used in milk

Chemical and physical methods and indirect instrumental methods

The content of FFAs (Peterková, 2002) is usually determined by BDI (titration of the isolated fat) and extraction-titration method (ČSN 57 0533, 1993). The above-mentioned conventional manual methods are different as compared by their results, little effective and therefore replaced by automatic instrumental determination of IR spectrometry (FT-MIR, mid-infra-red spectrum). This method needs adequate calibration according to the results of a reference method, which is BDI in this

case. It is necessary to determine the FFAs within 24 hours (reference method) or 48 hours (routine method), without preservation and at a storage temperature of milk samples below 5°C. Bijgaart (2006) and Broutin (2006) mentioned the FT-MIR procedure as relatively suitable in IDF material. It means a screening method where further investigations of other milk indicators are needed. Other methods were introduced in terms of an increase in their efficiency in FFA determination. For instance an enzymatic (acylation of coenzyme A by FFAs) segmented continuous flow method (Koops et al., 1990), which was compared with classical BDI with good results.

The MFPs were measured by two analytical methods. The first was carried out with the MilkoScan 6000 system (Foss Electric, Denmark). This was adjusted according to the results of a reference cryoscopic method in regular intervals. It means an alternative measurement of the milk freezing point equivalent. The other measurement procedure was performed by the own cryoscopic method, which was the instrument Cryo-Star automatic Funke-Gerber (Germany). It was realized with that part of the analysed milk samples that showed suspicious values by the first measurement method. The selected measurement mode was referential Plateau Search in this case. The used instrument was under regular calibration by the standard NaCl solutions and was regularly subjected to the national analytical proficiency testing with successful results. The work was performed according to the standard ČSN 57 0538 (1999). The incidental interferential effects were monitored.

The other investigated MQIs, such as F, L, CP, CAS, DM, SNF, U, FFA, were measured with the MilkoScan 6000 instrument (Foss Electric, Denmark; FT-MIR = mid-infra-red spectrophotometric apparatus with mathematical evaluation of the whole IR spectrum by means of Fourier's transformations), which was regularly calibrated according to the results of a referential method (standard ČSN 57 0536, 1999; Gerber's method for fat content, Kjeldahl's method for crude protein content and polarimetric and gravimetric methods for lactose and SNF contents, according to the standard ČSN 57 0530, 1972; for U and FFA according to the direct ureolytic, photometrical and BDI method results). In the small trial the FFAs were measured with Lactoscope FT instrument, calibrated according to the results of BDI method (Delta Instruments, Netherlands). The SCC was determined with the Fossomatic instrument (Foss Electric, Denmark) according to the standard ČSN EN ISO 13366-3, 1998. Both the previously mentioned instruments were subjected to the relevant national proficiency testing with regularly good results. The TMBC was determined with the Bactoscan 8000 instrument (Foss Electric, Denmark) under similar conditions like with the previous apparatus. The Bactoscan instrument was regularly and continuously calibrated according to the results of the plate cultivation method for TMBC. The colonies of mesophilic bacteria species were counted after their growth for 72 hours of incubation at 30°C (ČSN 57 0101, 1964).

Microbiological cultivation methods

The other microbiological indicators (ČSN 57 0101, 1964 ; ČSN ISO 4832, 1995; ČSN ISO 6730, 1996) were also investigated in an accredited testing laboratory. All these indicators are expressed in CFU/ml: TRBC, PBC, CBC. The GTK-M Agar (Milcom Tábor) was used as a culture medium for PBC and TRBC determination. The VRLB Agar (Milcom Tábor) was used for CBC cultivation. The temperature/time combinations of the microbiological cultivation conditions were 6.5°C/10 days for PBC, 30°C per 72 hours after previous milk sample inactivation by heating at 85°C for 10 minutes for TRBC and 36°C/24 hours for CBC.

Statistical procedures

The validation of the large data set was carried out by determination of the discrimination limits for all the MQIs. These limit values were derived from mean values and measure of variability as $x \pm 1.96$ or $2.58 \times$ SD, which included 95% or 99% probability that the values belong to the data set. If it was not possible, e.g. due to a marked deviation of data distribution from the normal frequency distribution, another procedure including the application of a qualified estimation was chosen. The result shows that data filtration should not let through wrong, improbable values subsequent evaluation. Of course, the data sets will always comprise the values out of the legislative framework of standard milk quality. The possible occurrence of extremely low or high values (especially in MQIs such as TMBC, SCC, CBC, TRBC and PBC) was solved individually by their exclusion in view of the

Table 1. The main statistical characteristics of FFA content and other quality indicators in raw cow milk

	n	*x*	*xg*	SD	*vx*	*m*	*n*	*x*	*xg*	SD	*CV*	*m*
FFA	5 840	0.61	0.46	0.46	74.7	0.51	5 746	0.83	0.70	0.49	58.8	0.75
F	5 793	4.25		0.38	8.9	4.24	5 690	4.14		0.36	8.7	4.12
CP	5 791	3.52		0.19	5.5	3.52	5 706	3.45		0.19	5.6	3.45
L	5 800	4.95		0.11	2.1	4.96	5 705	4.88		0.11	2.3	4.89
DM	5 723	13.35		0.46	3.4	13.34	5 630	13.10		0.44	3.4	13.1
SNF	5 723	9.16		0.25	2.7	9.17	5 667	9.03		0.25	2.7	9.03
F/CP	5 756	1.21		0.11	8.7	1.2	5 669	1.20		0.11	8.9	1.19
CAS	5 797	2.71		0.12	4.5	2.71	5 707	2.66		0.12	4.6	2.67
WP	5 616	0.68		0.09	13.0	0.675	5 670	0.65		0.09	14.0	0.65
U	5 680	3.31		1.24	37.3	3.33	5 730	3.62		1.40	38.7	3.55
MFP	5 780	526.00		5.58	1.1	526.00	5 695	523.00		5.91	1.1	524.00
SCC	5 741	256.00	225.00	138.17	54.0	234.00	5 645	254.00	224.00	133.85	52.7	238.00
TMBC	4 631	51.00	25.00	143.75	284.2	23.00	4 446	44.00	22.00	131.30	296.6	21.00
CBC	2 190	144.00	34.00	518.12	360.1	20.00	2 149	115.00	32.00	271.08	235.8	20.00
TRBC	242	1 210.00	576.00	2679.69	221.4	500.00	231	1 160.00	521.00	3 225.02	278.0	500.00
PBC	60	4 983.00	2 577.00	6 753.51	135.5	2 000.00	54	12 315.00	3 520.00	29 218.00	237.3	2 000.00

n = number of observations; *x* = arithmetical mean; *xg* = geometrical mean; SD = standard deviation; *CV* = coefficient of variation; *m* = median

Table 2. The normality investigation of two monthly data sets (I = nonstandard and II = standard) on FFA content in the fat of bulk samples of raw cow milk

Standard FFA, file II					Nonstandard FFA, file I				
	a_3	a_4	normality			a_3	a_4	normality	
			a_3	a_4				a_3	a_4
November	0.51	2.47	no	no	November	1.86	9.25	no	no
December	–0.01	2.23	yes	no	December	1.65	9.03	no	no

normal $a_3 = 0$, $a_4 = 3.0$; no = $P \leq 0.05$; yes $P > 0.05$

previous development of the values in the existing data source.

The general statistical evaluation of the data sets was performed separately for the individual calendar months (November and December) because of the generally valid model of the monthly payment system for raw milk. The main statistical characteristics, such as arithmetical (x) and geometrical mean (xg), standard deviation (SD) and coefficient of variation (vx), were calculated for the monthly data sets. If necessary, the MQI (FFA, SCC and microbiological indicators in this case) data were logarithmically transformed ($\log_{10}$) before the evaluation of the main statistical characteristics and mutual relationships because of no presumption of the normal data frequency distribution (Ali and Shook, 1980; Raubertas and Shook, 1982; Shook, 1982; Reneau et al., 1983, 1988; Reneau, 1986; Wiggans and Shook, 1987; Meloun and Militký, 1994; Kupka, 1997).

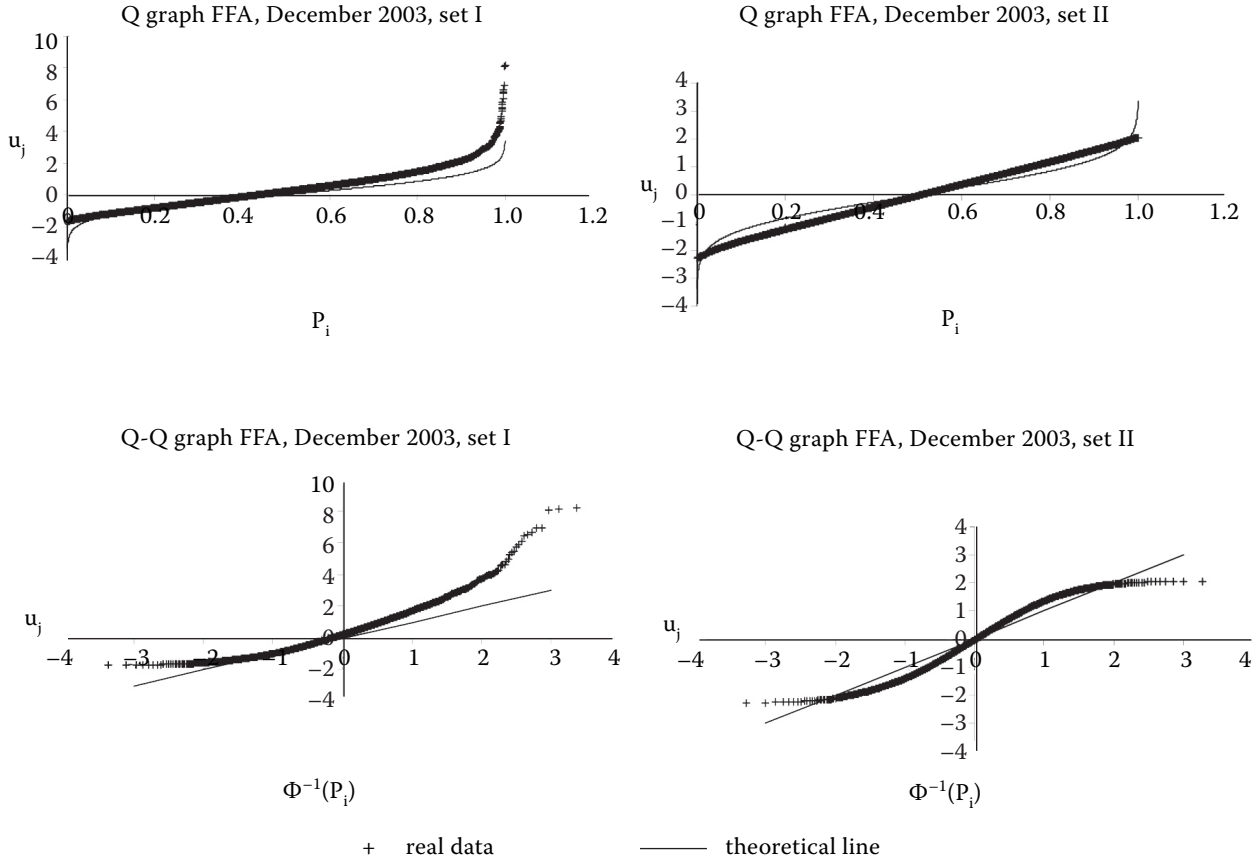

Figure 1. Exploratory analyse (Q = fractile and Q-Q = fractile-fractile graph) real frequency distribution of one-dimensional data file in comparison to standardized normal frequency distribution for FFA concentration in milk fat

Table 3. The normality investigation of two monthly data sets (I) on FFA content in the fat of bulk samples of raw cow milk using their logarithmic transformation

Nonstandard log FFA, 2003 I				
	a_3	a_4	normality	
			a_3	a_4
November	–0.93	4.78	no	no
December	–1.20	6.66	no	no

The regression analyses of mutual relationships between FFA *c*s and other MQIs were performed on the basis linear (LIN) and nonlinear models (such as logarithmical (LOG), power (POW), exponential (EXP), polynomial 2 (POL2) and 3 (POL3)). The evaluation was carried out in total data set (I nonstandard, NOV + DEC; *n* = as maximum 11 586 values of FFAs of milk fat). Results of FFAs in the small trial were evaluated by pair *t*-test criterion.

RESULTS AND DISCUSSION

Data set characteristics of FFA concentration in raw cow milk

The mean contents (file I) of free fatty acids in milk fat (Table 1) were 0.614 ± 0.458 mmol/100 g in November (*n* = 5 840) and 0.835 ± 0.491 mmol per 100 g in December (*n* = 5 746). It also means that a relatively high variability represented by the respective coefficients of variation 74.6% and 58.8% was found for bulk milk samples. The minimum and maximum values ranged from 0.005 to 4.172 mmol/100 g and from 0.007 to 4.859 mmol per 100 g, resp. The high coefficients of variation and the variation range explain the relatively significant difference between the arithmetic mean and the median (November 0.614 versus 0.510 and December 0.853 versus 0.752 mmol/100 g of both files (I = nonstandard) just as they confirm the fact that the adjustment of files (II = standard)

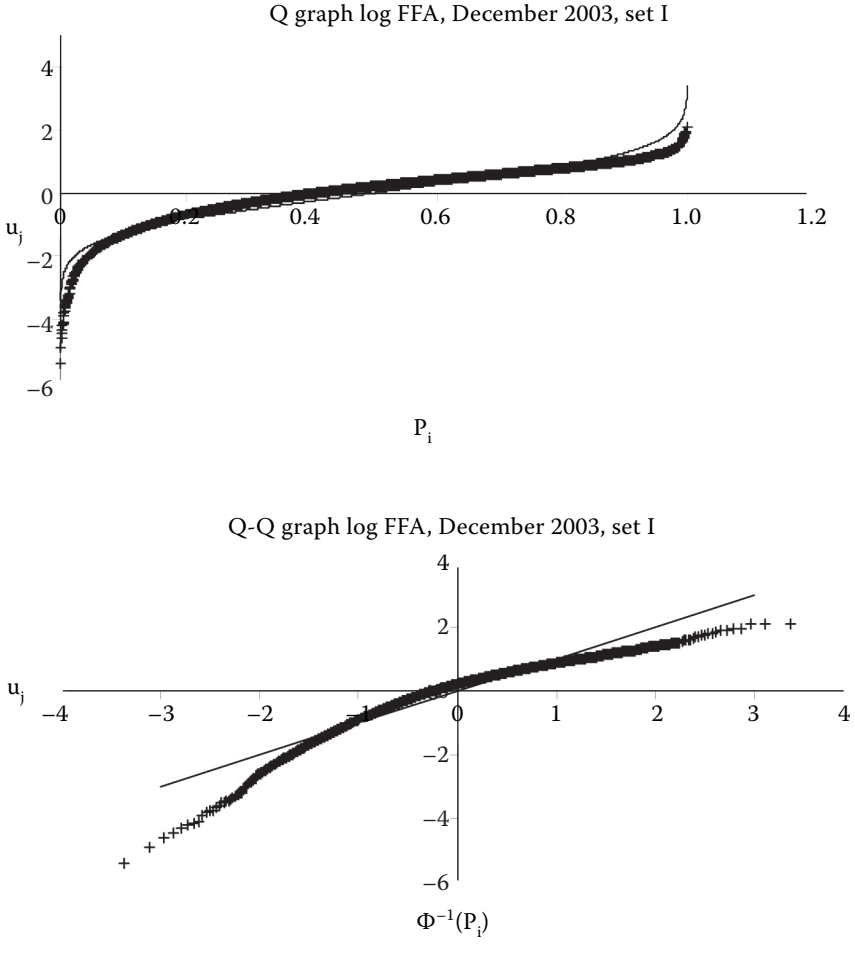

Figure 2. Exploratory analysis (Q = fractile and Q-Q = fractile-fractile graph) of the real freuency distribution of one-dimensional data set in comparison with standardized normal frequency distribution for log FFA concentration in milk fat

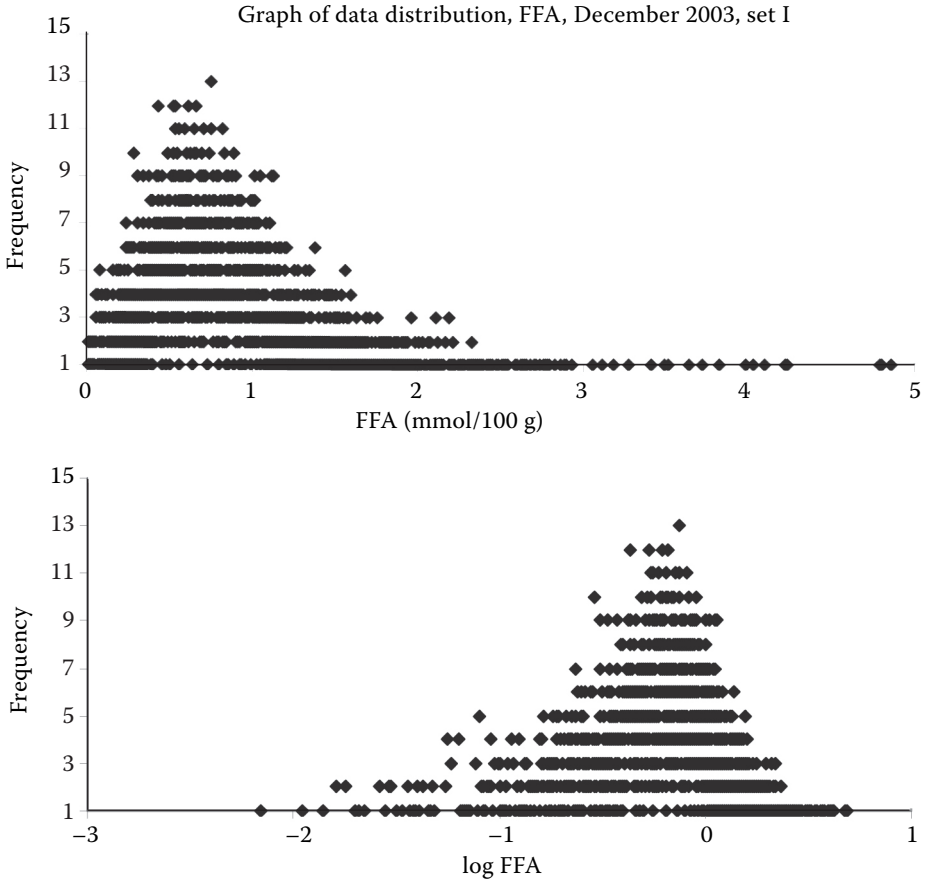

Figure 3. Frequency distribution of data set I on FFA in milk before and after log transformation

for nonstandard values (1.3 mmol/100 g) of FFAs standardizes their frequency distribution (Table 2; Figure 1). The skewness value (a_3) decreases from 1.86 and 1.65 to 0.51 and –0.01 (standard = 0). The acuteness value (a_4) is adjusted from 9.25 and 9.03 to 2.47 and 2.23 (standard = 3.0). A similar effect from the standpoint of standardization, but not so effective, was obtained after the logarithmic transformation of FFA values of file I (Table 3; Figures 2 and 3), where a_3 falls from 1.86 and 1.65 to –0.93 and –1.20 (standard = 0) and a_4 is adjusted from 9.25 and 9.03 to 4.78 and 6.66 (standard = 3.0). Nevertheless, it is probably more advantageous to use this method when evaluating the dependence of FFA content on the values of other milk quality indicators by regression procedure than the original data. As for the original (nonstandard) files, the frequency of occurrence of unsatisfactory FFA values varied more significantly and was 7.51% in November and 13.93% in December. Moreover, it was relatively quite high, which suggests a question about a need of further objective re-examination of the relevant standard discrimination limit for the purposes of raw milk quality evaluation under current conditions.

FFA concentration relations to the component and physical indictors of raw milk quality

From among the monitored milk component indicators the casein content was related to FFA *c* most closely where the correlation coefficient was –0.17 (Table 4; $P < 0.01$; Figure 4). Although it means that only 2.8% of FFA *c* variations can be explained by variability in the casein content, it still indicates that FFA *c* in milk fat can slightly increase with a decrease in the supply of nutrition energy to cows. The FFA *c* can increase by 0.066 mmol

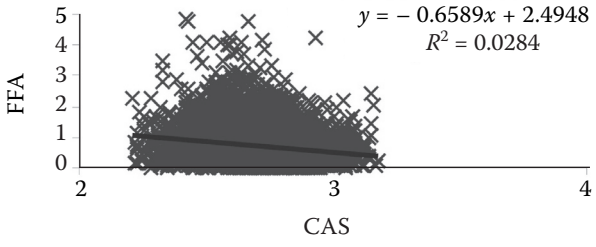

Figure 4. The linear regression relationship between casein content (CAS) in milk (%) and FFA concentration (mmol/100 g)

Table 4. Some linear and nonlinear regression relationships between milk quality indicators and FFA concentrations

Milk quality indicator	Regression type	Form of FFA	Regression equation	Determination coefficient R^2	Coefficient or correlation index r	
F	LIN	FFA	$y = -0.0569x + 0.9624$	0.0019	–0.04	NS
F	POL2	logFFA	$y = -0.0328x^2 + 0.231x - 0.6369$	0.0035	0.06	NS
CP	LIN	FFA	$y = -0.2861x + 1.7204$	0.0133	–0.12	**
CP	EXP	FFA	$y = 3.2628e^{-0.5037x}$	0.0157	0.13	**
L	LIN	FFA	$y = -0.6011x + 3.6763$	0.0202	–0.14	**
L	POL2	logFFA	$y = -0.6755x^2 + 6.1488x - 14.144$	0.0238	0.15	**
DM	LIN	FFA	$y = -0.1242x + 2.3645$	0.0142	–0.12	**
DM	POW	FFA	$y = 1\,227.7x^{-2.9773}$	0.0176	0.13	**
SNF	LIN	FFA	$y = -0.3036x + 3.4839$	0.0258	–0.16	**
SNF	LIN	logFFA	$y = -0.2267x + 1.8135$	0.0289	–0.17	**
F/CP	LIN	FFA	$y = 0.1444x + 0.5492$	0.0010	0.03	NS
CAS	LIN	FFA	$y = -0.6589x + 2.4948$	0.0284	–0.17	**
WP	LIN	FFA	$y = 0.1391x + 0.6329$	0.0007	0.03	NS
U	LIN	FFA	$y = -0.0292x + 0.827$	0.0063	–0.08	*
U	POL3	FFA	$y = 0.0102x^3 - 0.1144x^2 + 0.3553x + 0.4509$	0.0120	0.11	**
MFP	LIN	FFA	$y = 0.0043x - 1.5242$	0.0027	0.05	NS
MFP	POL3	FFA	$y = -7E\text{-}06x^3 + 0.0109x^2 - 5.7974x + 1\,029.2$	0.0090	0.10	**
SCC	LIN	FFA	$y = 0.0002x + 0.6818$	0.0022	0.05	NS
logSCC	POL3	logFFA	$y = 0.104x^3 - 0.6848x^2 + 1.5692x - 1.5057$	0.0044	0.07	*
TMBC	LIN	FFA	$y = 0.0002x + 0.6994$	0.0028	0.05	NS
logTMBC	LOG	FFA	$y = 0.1678\ln(x) + 0.6637$	0.0114	0.11	**
CBC	LIN	FFA	$y = 4E\text{-}05x + 0.727$	0.0011	0.03	NS
logCBC	POW	FFA	$y = 0.5173x^{0.2293}$	0.0107	0.10	**
TRBC	LIN	FFA	$y = -2E\text{-}05x + 0.6709$	0.0089	–0.09	NS
TRBC	EXP	FFA	$y = 0.5128e^{-4E\text{-}05x}$	0.0158	0.13	NS
PBC	LIN	FFA	$y = 8E\text{-}06x + 0.7972$	0.0750	0.27	*
PBC	LOG	FFA	$y = 0.1486\ln(x) - 0.3217$	0.0836	0.29	*

relationship significance NS = $P > 0.05$; * and ** = $P \leq 0.05$ and $P \leq 0.01$

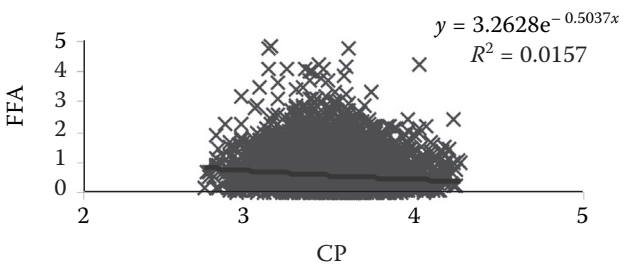

Figure 5. The linear regression relationship between crude protein content (CP) in milk (%) and FFA concentration (mmol/100 g)

per 100 g along with the casein content decrease by 0.10%. A similar relation corresponds with that in crude proteins in milk (Table 4; $r = -0.12$ or 0.13 resp.; $P < 0.01$; Figure 5) when this relation has been considered as a significant indicator of dairy cow energy nutrition level for a long time. The mentioned energy base can also confirm the FFA relation to lactose content, can increase along with the deteriorated health state of mammary gland (Peterková, 2002), consequently also with the decreasing lactose content (Table 4; $r = -0.14$ and 0.15 resp.; $P < 0.01$; Figure 6). As known, a significant negative relation exists between the lactose con-

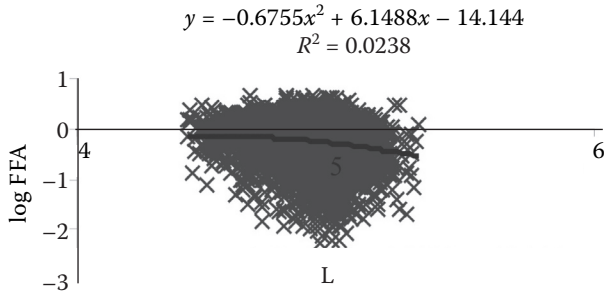

Figure 6. The linear regression relationship between lactose monohydrate content (L) in milk (%) and log FFA concentration (mmol/100 g)

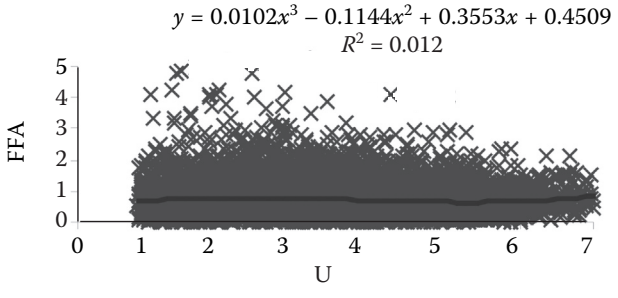

Figure 7. The linear regression relationship between urea concentration (U) in milk (mmol/l) and FFA concentration (mmol/100 g)

tent and somatic cell count as an indicator of the mammary gland health state (Hanuš et al., 1992, 1993a), nevertheless lactose can also be reduced by a significant deficiency of cow nutrition energy (Kirst et al., 1983, 1985) parallelly with the decreasing milk yield. Similarly, deficiency in the supply of nitrogen matters to dairy cows can be accompanied by a slightly higher FFA *c*, which indicates the urea and FFA *c* relation very weakly, $r = -0.08$ and 0.11 resp. (Table 4; $P < 0.05$ and < 0.01 resp.; Figure 7). The urea c in milk was previously been associated with the supply of nitrogen matters to dairy cows (Piatkowski et al., 1981; Hanuš et al., 1993; Homolka and Vencl, 1993; Jílek et al., 2006; Zhai et al., 2006). The fat content correlated with FFA *c* so weakly (Table 4) that it can be thought about a functional independence in tendencies and mutual physiological relations of these components, however so closely they may be to each other in chemical respect. MFP as a polyfactorial indicator was accompanied, in its better value, by an insignificant increase in FFA *c* (Table 4) ($P > 0.05$). The other significant negative correlations with FFAs were recorded for SNF (Table 4; Figure 8) and DM (Table 4). This means that FFA *c* can increase by 0.03 mmol/100 g with a simultaneous decrease in SNF content by 0.10%. Of all component indicators the milk nitrogen parts in particular were related significantly to FFA *c* in milk fat.

FFA concentration relations to hygienic indicators of raw milk quality

The SCC as indicators of the hygienic milk quality and the cow mammary gland health state, when it is known that the secretion disorders are connected with a higher FFA *c* (Peterková, 2002), correlated (Table 4; $r = 0.07$) with FFA *c* admittedly significantly ($P < 0.05$), nevertheless relatively weakly positively. The given relation thus explains only 0.4% of variations in FFA *c* through variability in SCC. The relation to another hygienic indicator, which is TMBC (Table 4; $r = 0.11$; $P < 0.01$), was positive and slightly closer and can explain 1.1% of FFA variability. FFA *c* correlated with coli bacteria occurrence similarly weakly (Table 4; $r = 0.10$; $P < 0.01$). FFA *c* correlated with the thermoresistant bacteria count admittedly insignificantly, however surprisingly negatively (Table 4). On the contrary, FFA *c* correlated with psychrotrophic bacteria more closely and significantly positively (Table 4; Figure 9; $r = 0.27$; $P < 0.05$). This

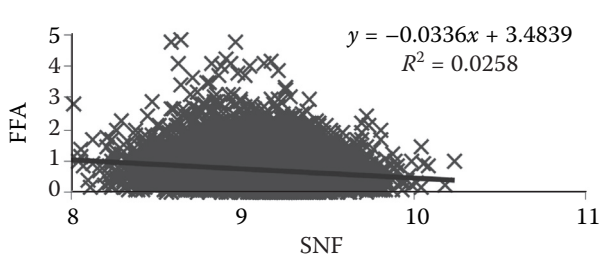

Figure 8. The linear regression relationship between solids-not-fat content (SNF) in milk (%) and FFA concentration (mmol/100 g)

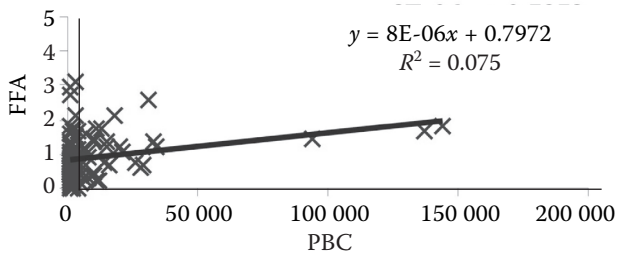

Figure 9. The linear regression relationship between psychrotrophic bacteria count (PBC) in milk (CFU/ml) and FFA concentration (mmol/100 g)

Table 5. The definition of mechanical stress

Indication	Conditions	Interpretation
P	Native milk	original milk, without mechanical stress
I.	+ 5 minutes M	small stress, comparable to milk treatment in primary production, milking, pipeline flow, pumping, mixing
II.	+ 10 minutes M	middle stress, primary production including processing technology, plus pumping, transport, centrifugation, homogenization, pipeline flow
III.	+ 15 minutes M	higher stress, conditions of strong stress in primary production and processing technology
IV.	+ 1 minute IRT	high stress, extreme conditions in primary milk production and processing technology
V.	+ 2 minutes IRT	very high stress, during the whole chain of primary production and processing technology including special preparation of milk drinks and food
VI.	+ 3 minutes IRT	extreme stress, experimental character only

the conditions of milk sample treatment = 0.5 l of milk; 36 minutes of mechanical stress in total; M = mixing (two-ply rotation stirrer, diameter 15 cm, 120 rot./minute); IRT = intensive rotation splintering – model situation (rotary with two blades, diameter 4 cm, 6 500 rot./minute)

means that FFA *c* increases by 0.006 mmol/100 g along with hygiene deterioration and PBC increase by 100 000 CFU/ml and that 8.4% of variations in FFA *c* can be explained by PBC variability. In general it can be stated, of course, that the deterioration of almost all hygienic indicators regularly meant FFA *c* growth, which contributes to the confirmation of multifactoriality of the discussed indicator.

The effect of mechanical milk stress on FFA concentration

Five bulk milk samples had the following indicators: *x*: F = 4.40%, CP = 3.38%, L = 4.91%; *xg*: SCC = 250 000 ml; TMBC = 25 300 CFU/ml; TRBC = 30.6 CFU/ml; CBC = 104.3 CFU/ml. The mechanical milk stress simulating the milking technology (Table 5) led to liberation of fatty acids from fat esters with intensity significantly proportional to the stress (Table 6). It was found that even a relatively small mechanical stress by mixing, comparable to the current milking technology, milk transport and storage increased FFA *c* of milk fat from 1.11 ± 0.19 (*CV* 17.1%) to 1.80 ± 0.40 mmol/100 g (i.e. by 0.69 mmol/100 g and by 62%; $P < 0.05$). The main increase then occurred with a stress that surely exceeds the milk routine treatment in the primary production. It can however be comparable with another milk stress accu-

Table 6. A test of the impact of mechanically induced lipolysis on FFA concentration (mmol/100 g)

Parameter	P	I.	II.	III.	IV.	V.	VI.
$\overline{x}$	1.11	1.80*	3.74**	5.08*	5.23^{NS}	5.65^{NS}	6.88**
SD	0.19	0.40	0.95	0.55	0.49	0.43	0.55
CV (%)	17.14	22.11	25.29	10.92	9.42	7.60	7.97
m	1.07	1.68*	3.49**	5.07***	5.36***	5.69***	6.88***
Max.	1.44	2.37	4.81	5.62	5.83	6.11	7.45
Min.	0.94	1.29	2.45	4.31	4.48	5.03	6.16
$R_{max.-min.}$	0.50	1.08	2.35	1.31	1.35	1.08	1.29

$n = 5$; $\overline{x}$ = arithmetical mean; SD = standard deviation; *CV* = coefficient of variation %; *m* = median; max. = maximum; min. = minimum; *R* = variation range; P = original milk; from I. to VI. = milk treatment by mechanical stress; difference significance (^{NS} = $P > 0.05$; *, ** and *** = $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$), at *x* from the previous type of treatment and at m from original milk

mulation caused by drawing, transport, skimming, homogenization and flow of another technology to the final product. It increased FFAs to 3.74 ± 0.95 mmol/100 g (*CV* 25.3%; $P < 0.001$). The highest experimental stress up to 6.88 ± 0.55 mmol per 100 g (*CV* 8.0%; $P < 0.001$), which means by 5.77 mmol/100 g (by 520%) as compared with the original *c*. Sjaunja (1984) also recorded a regular FFA *c* increase during repeated milk fat globule homogenization. Similarly, Vyletělová et al. (2000a,b) with increasing milk bacteria contamination during its storage.

CONCLUSION

From the standpoint of practical use the pieces of knowledge are important for possible modification of primary production factors by a preventive form for securing milk quality with a desirable FFA content and thus the animal health state and operation security of a farmer. Considering the technology milk quality securing and FFA *c* decrease in milk fat resp. and thus a durability extension of more demanding milk products it proves effective:

– to ensure satisfactory nutrition of dairy cows particularly in view of energy nutrient supply in such a way that no more marked decrease in crude protein and casein contents in milk can occur;
– to contribute to higher lactose contents in milk by preventing milk secretion disorders and negative energy balance;
– not to use fodder of deteriorated quality, e.g. partially rotten fodder with a high sporule content, and to reduce possibilities of secondary fermentation of supplied roughage feedstuffs;
– to avoid a disproportionate milk mechanical stress, e.g. as a result of milk column pulsation in the milk piping depending on negative pressure losses.

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