CHOSEN PARADOXES OF MAMMARY GLAND FUNCTION IN COWS AND RESULTS OF MILK PROPERTIES*

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Dairy paradoxes are the questions of relations between milk indicators (MI) such as fat content (F), urea concentration (U), somatic cell count (SCC), and milk freezing point (MFP). The aim of the study was to explain some paradox relationships. Relations of relevant MI in native milk and modified (F content change = MOD) samples were compared. Not even in an incorrect sampling or intentional technological milk modification such as MOD can it be thought of a possibility that it would come to a noticeable influencing of MFP (F × MFP; $r = 0.03$; $P > 0.05$) even though F is an important part of solid. Possible problems of a dairy herd with MFP (MFP × SCC; $r = -0.36$; $P < 0.05$ for native milk) cannot be mitigated by tolerance to higher SCC levels in bulk milk, either because of the risk of decrease of milk yield and quality. An incorrect milk sampling with an increased F content or its technological increase (dead milk) apparently damages milk quality in SCC (F × SCC; $r = 0.009$; $P > 0.05$ for native milk; but $r = 0.93$; $P < 0.001$ for MOD, where the F increase by 1% causes the SCC growth by 176×10^3 ml^{-1}).

cow; milk sample; fat; milk freezing point; urea; somatic cells

INTRODUCTION

(1) In explaining some relations in physiology of milk creation and its composition and properties, logical dependences can be certainly found out, but along with them explanation paradoxes, too. Knowing them grains importance in the effort to keep a good state of health and concurrently to achieve high milk yield and quality. The stated is also a current topic of the present milk production.

(2) Judging certain quality indicators of raw milk sometimes results in stating a paradox (sometimes apparent) deterioration of some quality indicators with improvement of the others. This possibility is given by the fact of numerous mutual links, dependences, and interference effects in multicomponent milk system in mammary gland lactogenesis. Sometimes the proper principles of dairy-analytical methods of measuring also contribute to the given effects. The correct milk quality interpretation presupposes knowledge of the substance of these phenomena. It often comes to antagonistic or apparently absurd findings in the link of health and composition milk indicators and physical properties as somatic cell count (SCC), urea concentration (U), milk freezing point depression (MFP), and fat content (F).

(3) There is not such a shortage of real or apparent paradoxes in biology or physiology and pathology of livestock. An example can be given known as „mammary gland immunity paradox" mentioned by Targowski (1985, quoted by Hejlíček et al., 1987). The thing is that it regularly comes to a markedly increased occurrence of new mastitis infections in cows essentially in the colostral period when the mammary gland is literally rich in antibodies for initiating passive immunity of the born calf. The said can be explained by creation of immunocomplexes arising in periods of increased permeability of the haematoalveolar barrier by antibody link to some protein components of mammary gland secretion (alfa-lactoglobulin, beta-lactalbumin and others). These immunocomplexes block the Fc receptors of phagocytes and so they decrease their phagocytar capacity very markedly. Obviously even leucotoxin secernated by mammary gland in the colostral period asserts itself. This toxin kills polymorphonuclears and

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macrophags as well. Understanding the mechanisms of "immunity paradox" of cattle mammary gland is a basic condition in the means construction for initiating an increased resistance of mammary gland against infection (Hejlíček et al., 1987).

(4) The SCC is a respected indicator of the state of health of mammary gland of mammal females from the standpoint of mastitis occurrence, milking quality, and hygienical milk quality from the standpoint of processing and entry into the foodstuff chain (Reneau, 1986; Sawa, Piwczynski, 2002; Jayarao et al., 2004; Golebiewski et al., 2011). The U in milk is a recognized indicator of nitrogen-energy balance (N/E) in the dairy cow nutrition in relation to their milk yield, when the increased values are often given by an absolute or relative nitrogen matters surplus or by energy lack (Homolka, Vencl, 1993; Jonker et al., 1999; Johnson, Young, 2003; Hojman et al., 2004; Jílek et al., 2006; Miglior et al., 2006). The MFP is a physically technological indicator for an intentional or unintentional, random or unavoidable foreign watter addition into milk, but today still more often also an indicator of the state of health of cow from the standpoint of N/E nutrition balance. Breed, season or milk yield influences are also referred to (Buchberger, 1990 a, b; Chládek, Čejna, 2005; Heck et al., 2009). The F content in milk is influenced, as known, chiefly by the fibre content in the fodder ration, by a solid proportion from concentrates in the solid of the overall fodder ration and possibly by acidose or by breed and dairy cow lactation stage or season, and in the ratio to milk proteins it is also considered as an indicator of suitability for cheesemaking (Agabriel et al., 1991; Hanuš et al., 2011a). For this reason it is now often used for interpretation of possible errors in dairy cow nutrition.

(5) Milk paradox as the object of this study could be defined as a contradictory result in terms of milk biology, chemistry or quality evaluation, for instance the improvement of one milk quality indicator along with deterioration of the others. Sure underestimation of paradox result existence could lead to mistake conclusions at evaluation of causes of some aggravated state or dairy troubles. Explanation of probability of such inconsistent result occurrence should protect against dairy interpretation mistakes. Therefore, the aim of this work was to provide experimental bases and rational explanation to some chosen detected milk compositional paradox situations and relations in lactogenesis physiology and in special cases of milk sample preparation in terms of milk analytical work.

MATERIAL AND METHODS

Animals and sample material

The sample material for the study of the placed problem was formed by four sample files, in which, besides chemical analyses, also other identification data such as cow breed, sample character, sampling season and all that were monitored:

(I) bulk milk samples (BMSs; *n* = 36) from a small number of cows (4–8 in a sample) of Holstein (H) breed in the first half of lactation (> 1st) in the period February–May, application of total mixed ration (TMR; maize silage 15 kg, alflafa silage 10 kg, maize spindle silage (LKS) 5 kg, brewery draff 3 kg, alfalfa hay 1 kg, dried whey 0.3 kg, and concentrates according to milk yield (MY) with yeast culture addition 5 kg per cow and day), free cowshed, milking in milking parlour with Fullwood tandem equipment, as sampling equipment electronic Fullwood flowmeters (Ellesmere, UK) were used, MY 28.11 kg per day;

(II) BMSs (*n* = 40) from a small number of cows (4–8 in sample) of Czech Fleckvieh cattle (CF) breed in the first half of lactation (> 1st) in the period from April to June, application of TMR (maize silage 13 kg, rye-grass silage 9 kg, LKS 5 kg, brewery draff 3 kg, concentrates according to MY 6 kg per cow and day), tie cowshed, pipeline milking equipment, as sampling equipment classical milk Tru-Test (Auckland, New Zealand) flowmeters were used, MY 26.17 kg per day;

(III) BMSs (*n* = 12) monthly all the year round mixed from half proportions of two herds (H and CF), the fat content modification was performed by refrigerator dead milk and separation (subsamples B, C) or by addition (D, E) of fat proportion to the original A milk. The subsamples with a modified F content then shared the otherwise "identical milk matrix". The process simulated thus an incorrect milk sampling, technological milk modification in the dairy and/or a deliberate manipulation with F used in creation of reference and pilot samples in milk laboratories in the quality control system of analytical work;

(IV) individual milk samples (IMSs) of H and CF breed (½ : ½, *n* = 960) from a three-year monitoring in the whole herd profile with respect to lactation order and stage. The sampling was always performed from selected dairy cows twice in summer and twice in winter periods in seven milk herds with MY from 5 500 kg (CF) to 10 000 kg (H) per lactation. Seven herds were sampled, three with H breed and three with CF breed, one herd was mixed (H and CF).

Analytical methods and resulting statistical evaluation

Samples were transported immediately under cooling conditions (< 10ºC) to the laboratory and analyzed in unpreserved state. The fat content (F) was determined using a Milko-Scan 133 B equipment (Foss Electric; Hilleröd, Denmark), which was calibrated according to the reference method. The urea concentration (U) in milk was measured by the photometric method after being dyed by para-dimethylaminobenzaldehyde (420 nm) on a Spekol 11 (Carl Zeiss; Jena, Germany). The somatic cell count (SCC) in milk was determined

Table 1. Main statistical characteristics of files I (H) and II (CF) of bulk milk samples

Breed	H			CF		
Statistics	$x \pm$ sd	xg	vx	$x \pm$ sd	xg	vx
F	4.06 ± 0.42		10.3	3.71 ± 0.46		12.3
U	40.4 ± 5.4		13.4	30.8 ± 5.4		17.4
SCC	141 ± 58	131	41.1	281 ± 212	216	75.4
MFP	–0.5320 ± 0.0050		0.9	–0.5202 ± 0.0043		0.8

H = Holstein, CF = Czech Fleckvieh, F = fat content (g 100 g^{-1}; %), U = milk urea concentration (mg 100 ml^{-1}), MFP = depression of milk freezing point (ºC), SCC = somatic cell count (10^3 ml^{-1}), $x \pm$ sd = arithmetic mean ± standard deviation, xg = geometric mean, vx = variation coefficient (in %)

Table 2. Main statistical characteristics of file III of fat content modified milk samples

Subsamples	C		E		A	
Statistics	$x \pm$ sd	xg	$x \pm$ sd	xg	$x \pm$ sd	xg
F	1.68 ± 0.12		6.48 ± 0.79		3.93 ± 0.27	
U	37.0 ± 11.0		36.0 ± 11.6		36.8 ± 11.0	
SCC	9.3 ± 2.9	9	812.0 ± 400.5	742	407.6 ± 188.7	380
MFP	–0.5218 ± 0.0086		–0.5236 ± 0.0076		–0.5234 ± 0.0089	

C, E = fat content modified milk samples, A = samples of native milk, F = fat content (g 100 g^{-1}; %), U = milk urea concentration (mg 100 ml^{-1}), MFP = depression of milk freezing point (ºC), SCC = somatic cell count (10^3 ml^{-1}), $x \pm$ sd = arithmetic mean ± standard deviation, xg = geometric mean

by fluorooptoelectronic reading on a Fossomatic 90 (Foss Electric, Hilleröd, Denmark). The milk freezing point (MFP) was determined on a Cryo-Star automatic (Funke-Gerber, Berlin, Germany). The measurements were performed in accredited laboratory (No. 1340, accred. cert. No. 040/2005) in conformity with EN ISO 17025 (including ISO 9002) standard. There were calculated basic statistics of data files of the milk indicators (MI; I, II, III, and IV) as arithmetic mean, standard deviation, variation coefficient. The SCC as indicator with a usually lognormal frequency distribution was evaluated in original values and also in values logarithmically transformed ($\log_{10}$) for calculation of geometric means. Linear, possibly non-linear regression analyses were performed among the appropriately corresponding files of MI, and coefficients, possibly correlation indexes and determination coefficients, were calculated. The subsample files (III, A = native milk, B, C, D, and E = technologically modified milk with fat content changes) were demonstrated by box charts.

RESULTS AND DISCUSSION

Basic characteristics of data files

(1) The averages and variability of native MI (F, U, SCC, and MFP; BMS, files I (H) and II (CF)) showed values which are presented in Table 1. The presented mean values of MIs and their variability as well were within the frame of the usual state as compared to results by J a n š t o v á et al. (2011), a little lower in SCC as compared to B e r r y et al. (2006) and comparable as compared to H e c k et al. (2009), lower (better) in MFP as compared to H e c k et al. (2009), but higher in U as compared to results by H e c k et al. (2009) (24 ± 9 mg per 100 g) and G o l e b i e w s k i et al. (2011) (25.1 mg per 100 ml).

(2) The fat manipulations (file III) changed the F significantly, relatively by –57.3% and 64.9% and it ranged from C to E with a mean and variability of native milk A (Table 2, Fig. 1). The corresponding means and variability of the subfiles of subsamples C, E, and A for the mentioned MIs like U, SCC, and MFP are given also in Table 2. Values are well comparable to our previous results with initial experiment by H a n u š et al. (2003), however, lower (better) in MFP as compared to results by Heck et al. (2009) (–0.519ºC). The U, SCC, and MFP indicator changes corresponded to the performed fat content modifications. The deteriorated raw milk freezing point was here a relevant problem of milk payment of the last period. It has been proved by various methods that despite the existence of opposite opinions the MFP gets worse significantly with the growing MY particularly in H breed, and thus probably also in the long term with the breeding for MY (Janů et al., 2006).

(3) In file IV the mean values were for SCC 217 ± 528 10^3 ml^{-1} (lower as compared to Golebiewski et al. (2011) (xg = 76 10^3 ml^{-1}; vx = 243%)) and for F 3.95 ± 0.90% (vx = 22.8%).

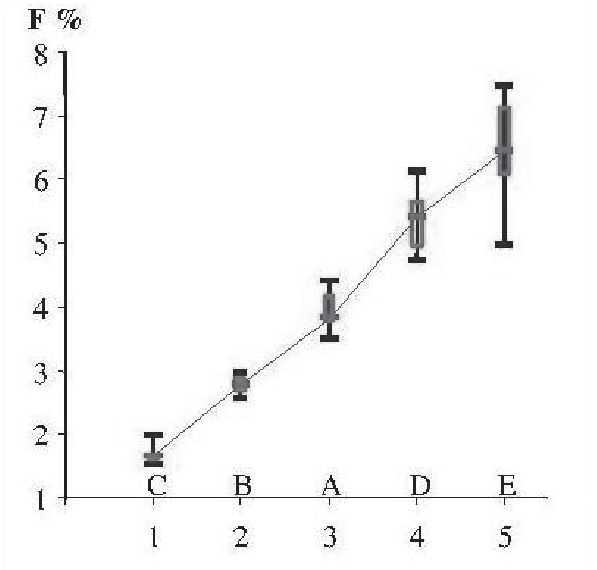

Fig. 1. Native (A) fat content (F) in raw cow milk and after its content manipulation (C, B, D, E)

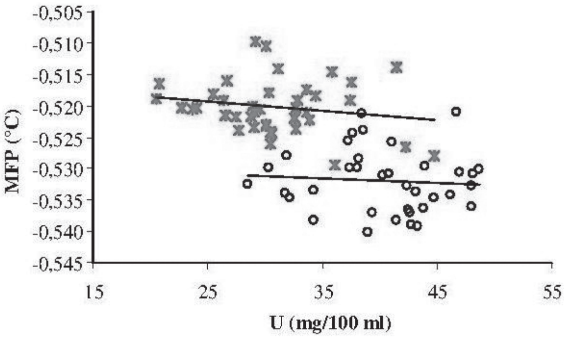

Fig. 2. Dependence of milk freezing point (MFP) on urea (U) concentration in cow milk (ºHolstein; × Czech Fleckvieh). Correlation r = -0.19 ns (for ×) R^2 = 0.0361 and -0.08 ns (for º) R^2 = 0.0064; Statistical significance: ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

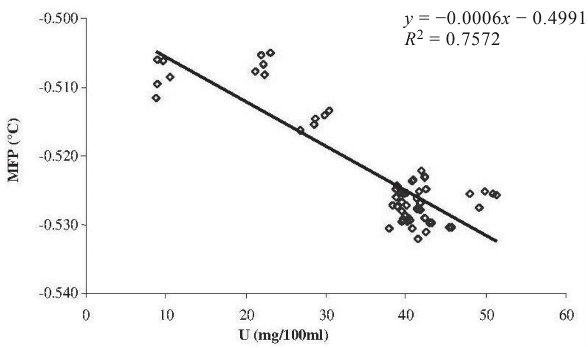

Fig. 3. . Dependence of milk freezing point (MFP) on urea (U) concentration in cow milk after fat content manipulation..
r = –0.87***

Relation of freezing point and urea concentration in milk

The MFP and U relation was negative (Fig. 2) (file I (H) and II (CF)) (r = –0.08 and –0.19, respectively) due to the osmotically strongly active character of U, nevertheless, insignificantly (P > 0.05). This suggests and the following relation confirms it that U participates by about 1.5–2.5% in the MFP (B u c h b e r g e r , 1994; C r o m b r u g g e , 2003). The fat content modifications in native identical milk, which under the influence of the changed conditions of specific weight of fat and aqueous milk phases resulted in a moderate growth (from 36.0 ± 11.6 to 37.0 ± 11.0 mg 100 ml^{-1}) by 1 mg 100 ml^{-1} of U with a F decrease (from E to C), showed a close relation between MFP and U in milk (r = –0.87; P < 0.001) (Fig. 3). The introduced for this investigation case suggested that 75.7% of variations in MFP had been caused by variations in U, which is a relatively high value. It implies practically and logically, of course, that the higher U in milk, the better the MFP and thus apparently also milk quality is. At the same time, however, a higher U in natural milk samples is an indicator of a higher nitrogen loading of a dairy cow in nutrition or of a lower supply of her metabolism with energy (B a k e r et al., 1995; H o j m a n et al., 2004) and a by-product, if it is of a more permanent character of a worse fertility (P i a t k o w s k i et al., 1981; B u t l e r et al., 1996). Ř í h a , H a n u š (1999) and H a n u š et al. (2000) found that a longterm increase of U in milk by 10 mg 100 ml^{-1} above a usual average was connected with prolongation of the service period by 10 days. H o j m a n et al. (2004) reported that a significant negative relation was found between the milk U level and the conception rate which was 38.4% for the lowest quartile of milk U and 36.1% for the highest quartile. K u b e š o v á et al. (2005, 2009) found important relations of U in milk to the service period and insemination interval length, r = 0.26 and 0.37. Also B e r a n et al. (2012) found out that higher values of milk acetone and urea negatively affected sperm survival during the short-term heat test. In the same way too low or high U levels can have an impact on reduced longevity. H a n u š et al. (2000) reported that longterm U values under 25 mg 100 ml^{-1} were connected with a production age reduction by 0.48 of lactation and of the longterm value above 35 mg100 ml^{-1} with reduction by 0.65 of lactation (P < 0.01 and P < 0.001). Sometimes, however, a deterioration of cow fertility with a growing U nitrogen concentration in milk also was not recorded. Ř e h á k et al. (2009) did not find the effect of U on the probability of conception at the first service. The MFP improvement by tolerance of a higher U in milk cannot be therefore a successful practical solution of the possible problem. Energy sufficiency in the cow nutrition is needed owing to their MY so that the physiological U level in milk can be maintained without a negative impact on dairy cow state of health and at the same time an adequate MFP stabilized. Otherwise it is subject to deterioration in case of energy malnutrition (H a n u š et al., 2011b).

Relation of freezing point and fat content in milk

The F content and MFP as milk quality indicators should not be related too closely, though a closer relation could be presupposed. Various studies (K o o p s et al., 1989; B u c h b e r g e r , 1994; C r o m b r u g g e , 2003) showed that the F content could participate by about three per cent in the cryopoint depression, when fat, proteins, peptides, and free amino acids receive about 7%. In spite of that, in the present general and permanent decrease of mean fat content in connection with a growing MY of cows in the Czech Republic, a suspicion arose saying that this tendency predominantly in high yield herds could be in connection with a likewise problematic MFP. This opinion was also supported by casual commercial problems with export of skimmed milk, when a deteriorated MFP was often declared. The correlative relations between the mentioned indicators in native BMSs (Fig. 4) (files I and II; $r = -0.46$ for CF and -0.19 for H) were contradictory with the previous facts, relatively significant in CF breed ($P < 0.01$). This relation, of course, could have been given by other components related to MFP, e.g. by overall solid. Evidently for that reason the presented relation in H breed was considered as insignificant ($P > 0.05$). In performing marked fat manipulations in identical milk, which included mean decrease and growth of F content by 2.25 and 2.55% (Fig. 1), the MFP did not almost change (Fig. 5) (file III; $r = 0.03$; $P > 0.05$), similarly as in the previous work (H a n u š et al., 2003). The F content as a substantial milk solid component almost does not affect MFP paradoxically, and if its level is technologically altered, then the possible minimum impacts on MFP arise rather from the concurrent change of matter specific weight, i.e., from concentration of the other solid components with F decrease. Further, the presented also confirms that the fat skimming as a usual technological process could not have been the cause of the commercial problems with MFP.

Relation of freezing point and somatic cell count in milk

In the regression relations inside the native BMSs a decrease or improvement of MFP, respectively, with SCC growth can be observed paradoxically, as if a deteriorating state of health of the herd from the point of view of mammary gland (Fig. 6) (files I and II; $r = -0.36$; $P < 0.05$). The correlation was relatively close in particular in breed H and referred to the fact that up to 13.0% of variations in MFP could be determined by variations in log SCC. In CF breed a similar tendency with a slighter closeness was observed. In general, in accordance with this introduced fact also H e c k et al. (2009) mentioned higher (worse) MFP in BMSs (–0.519ºC) at lower SCC (186 10^3 ml^{-1}). Nevertheless, reasons for this fact could be a little bit different, for instance high milk yield of Dutch dairy herds (for MFP). While it was so in native samples, the mentioned tendency in modified samples did not show itself and was even contrary (Fig. 7) (file III; SCC × MFP; $r = 0.30$; $P < 0.05$; log SCC × MFP; $r = 0.07$; $P > 0.05$). The stated contradiction confirms the hypothesis, for it was here a case of an identical milk matrix, and salts as other solid components were concentrated only moderately owing to the specific

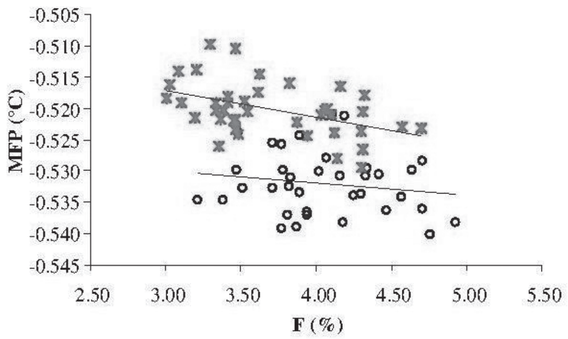

Fig. 4. Dependence of milk freezing point (MFP) on fat (F) in cow milk (º Holstein; × Czech Fleckvieh)
r = –0.46** (for ×) $R^2 = 0.2116$ and r = –0.19 ns (for º) $R^2 = 0.0361$

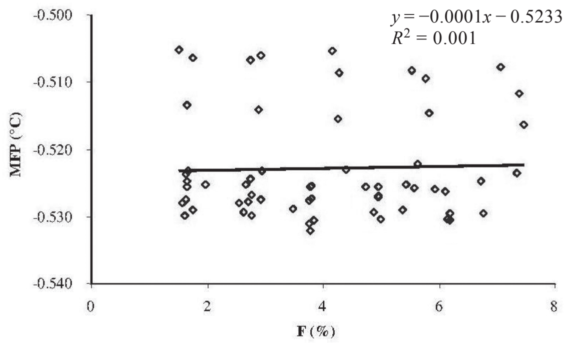

Fig. 5. Dependence of milk freezing point (MFP) on fat (F) in cow milk after fat content manipulation
r = 0.03 ns

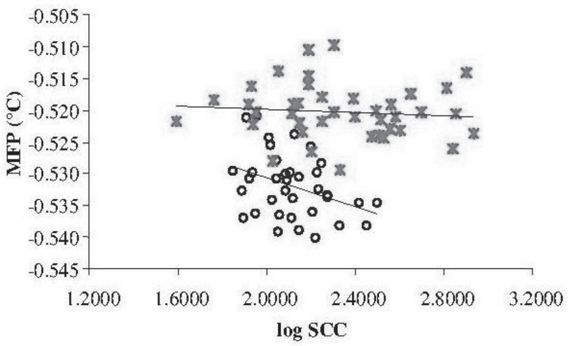

Fig. 6. Dependence of milk freezing point (MFP) on somatic cell count (SCC) in cow milk (º Holstein; × Czech Fleckvieh
r = -0.36* (for º) $R^2 = 0.1296$

weight growth due to the manipulations with fat content or its decrease, respectively. The mentioned results can be explained by the fact that during lactose content decrease in milk due to the damage of mammary gland secretory epithelium with development of e.g. subclinical mastitis, it comes with SCC growth to an increased secretion of osmotically active Na^+, K^+, and also Cl^- ions for the physiological ability of milk production to remain preserved. The increase of salt concentration thus can result even in MFP improvement. Paradoxically, the MFP can be therefore improved with SCC growth according to the character of milk secretion disorder or mastitis.

Relation of fat content and somatic cell count in milk

The fat content relation to SCC was formulated as a very weak, almost zero correlation coefficient (Fig. 8) (file IV; $r = 0.009$; $P > 0.05$; $n = 960$) in the usual set of cow IMSs from the whole lactation and herd profile from the standpoint of the lactation order. A similar relation can probably be also valid in the files of ter-

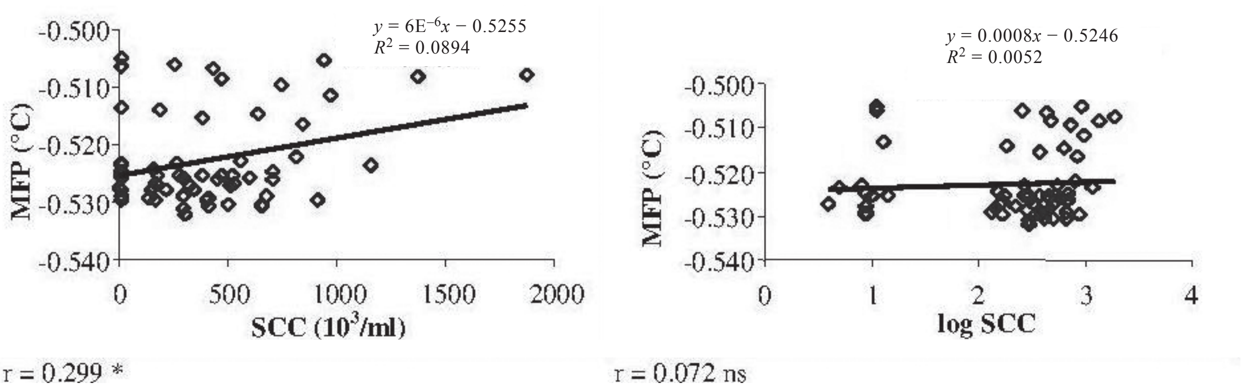

Fig. 7. Dependence of milk freezing point (MFP) on somatic cell count (SCC) in cow milk

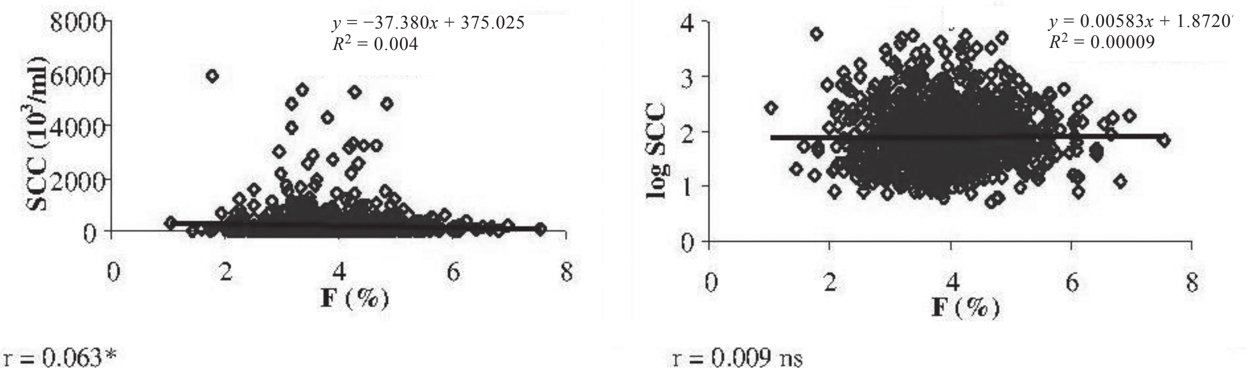

Fig. 8. Dependence of somatic cell count (SCC) on fat (F) content in cow milk

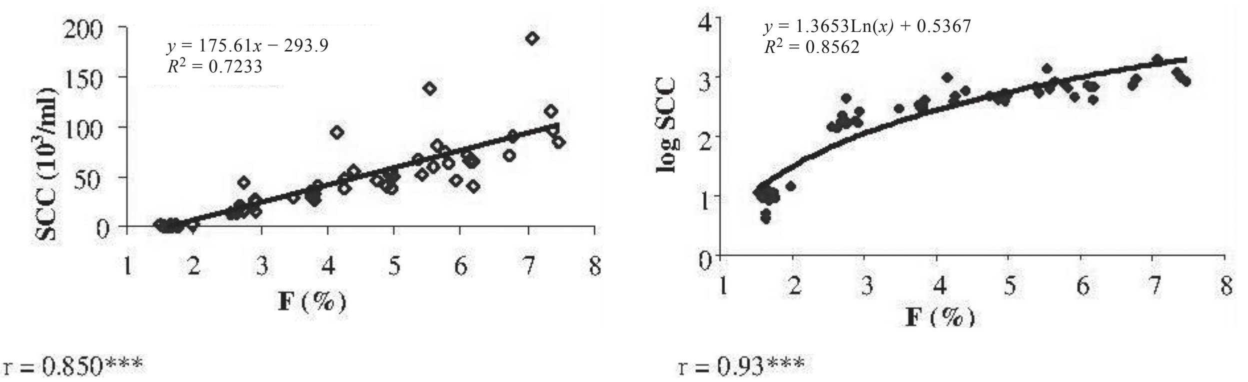

Fig. 9. Dependence of somatic cell count (SCC) on fat (F) content after manipulation in cow milk

rain BMSs where J a y a r a o et al. (2004) reported positive medium and higher significant correlations of SCC first of all to various bacterial and pathogen milk contamination in accordance with general quality indication ability of SCC in milk. Nevertheless, a much closer positive relation is detected when the fat content is modified randomly or deliberately or manipulated, respectively (Fig. 9) (file III; logarithmic regression $r = 0.93$; $P < 0.001$), (Fig. 10) (difference $P < 0.001$). Here 85.6% of variations in log SCC could be explained by artificial variations in fat content (Fig. 1). As evident, the noticeable growth and decrease of F content (Fig. 1) resulted in distinctive SCC growth and decrease (Figs. 9, 10), likewise as in the previous work (H a n u š et al., 2003). It follows then from the linear regression of the same F × SCC relation (Fig. 9) ($r = 0.85$; $P < 0.001$) that the F growth by 1% (Fig. 1) can cause the SCC growth by 176 10^3 ml^{-1}. Therefore the relation of high SCCs to the high F contents in the usual files of native milk samples is not in particular a reflection of milk secretion physiology except the case of milk, which was obtained to the end of longer lactation, especially the files of IMSs, when the F content growth is usually accompained even by higher SCCs, than rather a reflection of possible sampling errors or involuntary or intentional compositional manipulations, respectively. In practical monitoring of milk quality, e.g., for the quality payment purposes (files of BMSs) it is therefore essential to eliminate the analyses results of such samples with concurrently markedly increased F and SCCs. The phenomenon is explained hypothetically by an increased adhesion between the F globules and the somatic cells, which are then transferred by physical cell effect into the fat fraction. This is facilitated due to a higher volume impact of individual fat droplets in comparison to cells and due to the approximately balanced minus divergence of fat specific weight and plus divergence of cell material specific weight from value one. The SCC grows therefore paradoxically in a fatter proportion of milk during skimming or dead milk. It is also important to take that into account in laboratories manipulating with milk components methodically with intention in order to prepare reference calibration or pilot samples for the quality control of the dairy analyses results.

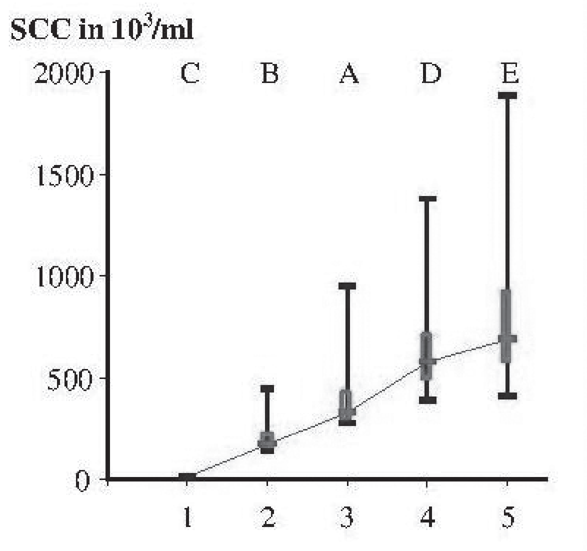

Fig. 10. Dependence of somatic cell count (SCC) on fat content in native cow milk (A) and after fat content manipulation (C, B, D, E; according to Fig. 1.)

CONCLUSION

The individual experimentally observed spheres can result in the following conclusions:

- the worse nutrition state endangering cow reproduction connected with a higher nitrogen loading of cow metabolism and with the increased milk U levels can paradoxically "improve" milk quality in terms of MFP due to the U osmotic activity, which, however, cannot be a reason of tolerance for this possibility of development in a milked herd;
- not even in an incorrect sampling or a deliberate technological adjustment of milk in terms of F content modification is it possible to think of a possibility that it would come to an observable influence of MFP, even when F is an important component of milk solid;
- possible problems of dairy cow herds with MFP owing to a decrease risk of both animal MY and changes of the other milk quality indicators cannot be mitigated by benevolence or tolerance to higher SCC levels in bulk milk;
- incorrect milk sampling in terms of increased fat level is apparently harmful to raw milk quality primarily in the SCC indicator. Therefore a consistent control of the process corectness of milk sampling is of essential importance, and the practical systems should have this activity officially validated and accredited.

From the standpoint of practical use of the results it is advantageous to know the interpretation of the mentioned paradoxical phenomena for:

- preparation of reference and pilot samples for the dairy analytical apparatuses control and their achievement testing and evaluation of possibilities and mutual manipulation consequences with components within the named activity;
- identification and evaluation of possible incorrect milk sampling and its impact on the stated resulting quality;
- evaluation and estimate of the influence of possible indispensable technological compositional adjustments for modifications usual in milk processing;
- evaluation of the cow health state and possible effective factors of its change from the proportion in-

terpretation of the MI within the prevention of animal production disorders.

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