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A COMPARISON OF RESULT RELIABILITY FOR INVESTIGATION OF MILK COMPOSITION BY ALTERNATIVE ANALYTICAL METHODS IN CZECH REPUBLIC

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Abstract

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The milk analyse result reliability is important for assurance of foodstuff chain quality. There are more direct and indirect methods for milk composition measurement (fat (F), protein (P), lactose (L) and solids non fat (SNF) content). The goal was to evaluate some reference and routine milk analytical procedures on result basis. The direct reference analyses were: F, fat content (Röse–Gottlieb method); P, crude protein content (Kjeldahl method); L, lactose (monohydrate, polarimetric method); SNF, solids non fat (gravimetric method). F, P, L and SNF were determined also by various indirect methods: – MIR (infrared (IR) technology with optical filters), 7 instruments in 4 labs; – MIR–FT (IR spectroscopy with Fourier's transformations), 10 in 6; – ultrasonic method (UM), 3 in 1; – analysis by the blue and red box (BRB), 1 v 1. There were used 10 reference milk samples. Coefficient of determination (R^2), correlation coefficient (r) and standard deviation of the mean of individual differences (MDsd, for n) were evaluated. All correlations (r; for all indirect and alternative methods and all milk components) were significant ($P \leq 0.001$). MIR and MIR–FT (conventional) methods explained considerably higher proportion of the variability in reference results than the UM and BRB methods (alternative). All r average values (x minus 1.64 × sd for 95% confidence interval) can be used as standards for calibration quality evaluation (MIR, MIR–FT, UM and BRB): – for F 0.997, 0.997, 0.99 and 0.995; – for P 0.986, 0.981, 0.828 and 0.864; – for L 0.968, 0.871, 0.705 and 0.761; – for SNF 0.992, 0.993, 0.911 and 0.872. Similarly MDsd (x plus 1.64 × sd): – for F 0.071, 0.068, 0.132 and 0.101%; – for P 0.051, 0.054, 0.202 and 0.14%; – for L 0.037, 0.074, 0.113 and 0.11%; – for SNF 0.052, 0.068, 0.141 and 0.204.

Keywords: cow raw milk, milk composition, reference method, indirect method, adjustation, analytical result reliability

INTRODUCTION

Assurance of good health state of farm animals is always more important for support of foodstuff chain safety. There is whole row of components in milk which are able to contribute to control the cow health state via method of noninvasion monitoring. Especially, there are so called majority components: fat (F); protein (P); lactose (L); somatic cell count (SCC). These values and their combinations could be important for control

of prevention of subclinical forms of production disorders such as mastitis during whole lactation (milk yield, conductivity, L and SCC; Hanuš *et al*., 1992; Pyorälä, 2003; Katz, 2007; Karp and Petersson Wolfe, 2010) and ketosis in early lactation (milk yield, F/P and F/L; Steen *et al*., 1996; Geishauser *et al*., 1997; Duffield, 2000; Katz, 2007; Duffield *et al*., 2009; Siebert and Pallauf, 2010; van der Drift *et al*., 2012; Hanuš *et al*., 2013). Milk in contrast to blood or urine offers easy sampling, which is routinely mastered including cold transport into laboratory. The quality of analytical results decides about rightness of their practical interpretation and about efficiency of prevention or treatment measures in herds and also about possibilities of foodstuff milk chain quality assurance. Therefore, the systems of analytical quality assurance (AQA; CSN EN ISO 17025; Grappin, 1987, 1993; Baumgartner, 2006; Hanuš *et al*., 2007, 2011 a; Leray, 2009 a, b; Barbano, 2009; Castaneda, 2009) and proficiency testing (PT; Wood, *et al*., 1998; Golc Teger, 1996, 1997; Coveney, 2001; Hanuš *et al*., 2007; Leray, 2010) are built in accredited milk laboratories according to relevant standards (CSN 57 0530; CSN 57 0536; CSN EN ISO/IEC 17025). Beside reference methods there is row of indirect methods in the milk analyse. Their number and expansion is growing up along increase of demands on animal health and milk product quality: infrared (IR) spectrometry in mid (MIR and MIR–FT; Baumgartner, 2006; Hanuš *et al*., 2007, 2008, 2011 a; Leray, 2009 b; Barbano, 2009; Castaneda, 2009; van der Drift *et al*., 2012) and near (NIR; Kukačková *et al*., 2000; Jankovská and Šustová, 2003; Šustová *et al*., 2007) IR range (for F, P, L, solids non fat (SNF)); photocolorimetry (P; amidoblack 10 B); ultrasonic method (F, P, L, SNF; Perlín, 2003); nephelometry (F).

The goal of this study was to evaluate some reference and routine milk analytical procedures and carry out mutual comparison of their results. Today, the above mentioned various methods are used in milk laboratories at universities, dairy plants, agricultural enterprises and in milk recording. Therefore, information about comparison of result reliability is important for laboratory staff.

MATERIAL AND METHODS

Used Dairy Analytical Procedures

The direct reference (REF) analyses carried out including abbreviations and units of measurement were as follows: F, fat content (according to Röse–Gottlieb extraction and gravimetric method; %); P, crude protein content (according to Kjeldahl method by mineralization, steam distillation and titration, total N × 6.38, %); L, lactose (monohydrate %; by polarimetric method); SNF, solids non fat (%; by gravimetric method for content of total solids without F).

F, P, L and SNF in milk were determined also by various indirect methods mostly in experimental localities (included laboratories) in the Czech Republic but also in the Slovak Republic: – MIR (infrared (IR) technology with optical filters), 7 instruments in four laboratories; – MIR–FT (IR spectroscopy of whole spectrum by Michelson's interferometer and use of Fourier's transformations), 10 instruments in six laboratories; – ultrasonic method (UM, modification of ultrasound during its passage through milk by its organic molecules), 3 instruments in one laboratory; – analysis by the blue (nephelometry and impedance) and red (thermoanalyse) box (BRB), 1 instrument in one laboratory (Lactostar, 2005).

The analytical methods, calibration and operation of instruments were carried out in accordance with relevant standards (CSN 57 0530, 57 0536, CSN EN ISO 8968-1, CSN EN ISO 1211, CSN EN ISO 17025) and the manufacturer's manual.

Animals and Milk Samples

Overall, methods were evaluated in three sampling periods (3 × 10 samples) in winter feeding period (Dezember 2013, March and April 2014). In every case the bulk milk samples were collected from three dairy herds: 1) Holstein (H); 2) Czech Fleckvieh (CF); 3) H + CF. 10 reference samples were prepared from collected milk for each sampling period: 5 samples of the original native milk; 5 samples was modified with respect to the basic components of milk by procedures as previously described and validated (Hanuš *et al*., 2007, 2008, 2011 a, b). The set of reference samples (n = 10) showed a variation range of the main components that is necessary for calibration and its validation. The milk samples were refrigerated and preserved with bronopol (0.02%) for transport before measurement.

Statistic Treatment of Results

The basic statistical characteristics were calculated (Microsoft Excel) for individual data files (n = 10 measurements in the set). Also difference statistic and linear regression was performed. Linear regression model is the calibration basis of dairy analytical techniques (Baumgartner, 2006). What is important is the closeness of result relationships of compared methods. Shift on the axis can be easily corrected by calculation. Therefore, for this purpose we used the evaluation through: coefficient of determination (R^2); correlation coefficient (r); standard deviation of the mean of individual differences (MDsd, for n). These indicators are essentially independent on the shift of relevant calibration regression line and in practice relatively little affected by slope of this line (Sjaunja, 1984; Sjaunja *et al*., 1984; Sjaunja and Andersson, 1985). Therefore, these are suitable to objective assess the quality of the calibration.

Overall, the results of analytical methods were adjusted (adjustment of all sampling terms (3) to one sampling term) and compared (ranged) using Euclidean distance from origin (Ed) in proficiency

testing (PT model; Grappin, 1993; Golc Teger, 1996, 1997; Wood *et al.*, 1998; Hanuš *et al.*, 2007, 2008, 2011 a; Leray, 2009 a, b, 2010) according to milk components.

RESULTS AND DISCUSSION

The statistics of reference results (reference methods) of milk constituents (F, P, L and SNF) for sets (n = 3) of calibration samples are shown in Tab. I. In particular, at parameter of variation range this fact is seen that the reference sets were suitable for calibration purposes (CSN 57 0536). This is obvious that the maximum variation range is methodically and purposefully achieved in the fat. There are included native samples and samples with modified fat percentage. This procedure was justified and analyzed in the previous study (Hanuš *et al.*, 2011 b).

The relations between the reference and indirect methods for the 4 monitored milk components are shown in Tabs. II, III, IV and V. It can be stated that all obtained correlation coefficients (for all indirect and alternative methods and all components of milk) were statistically significant ($P \leq 0.001$; the average examples of linear regressions are selected in Figs. 1–4). In the Czech Republic dairy laboratory system it is also evident that the methods MIR and MIR–FT (conventional) explained on the average usually considerably higher

I: *Main values of composition of sets of reference (REF) milk samples for calibration of indirect methods*

	n	F		P		L		SNF	
		x ± sd	VR	x ± sd	VR	x ± sd	VR	x ± sd	VR
set 1	10	4.24 ± 0.95	2.49 5.30	3.42 ± 0.26	3.07 3.79	4.81 ± 0.14	4.47 5.01	8.79 ± 0.36	8.26 9.22
set 2	10	4.27 ± 1.14	2.22 5.66	3.27 ± 0.18	3.07 3.56	4.87 ± 0.16	4.53 5.04	8.68 ± 0.3	8.16 9.08
set 3	10	4.1 ± 1.01	2.17 5.33	3.23 ± 0.19	2.99 3.49	4.87 ± 0.14	4.54 5.07	8.7 ± 0.3	8.16 9.1

x ± sd aritmetic mean and standard deviation; VR variation range, from minimum to maximum; n number of samples; F fat (%); P crude protein (%); L lactose (%); SNF solids non fat (%)

II: *Expression of the relationship and the potential for calibration quality of indirect methods according to reference values (method) of milk fat (F)*

Instrument, method	n	R^2		r		MDsd	
		x ± sd	VR	x ± sd	VR	x ± sd	VR
MIR	7	99.82 ± 0.25	99.21 99.98	0.999 ± 0.001	0.996 0.9999	0.035 ± 0.022	0.014 0.084
MIR–FT	10	99.9 ± 0.11	99.64 99.98	0.999 ± 0.001	0.9982 0.9999	0.038 ± 0.018	0.014 0.065
UM	4	98.78 ± 0.4	98.29 99.41	0.993 ± 0.002	0.9914 0.997	0.107 ± 0.015	0.086 0.12
BRB	4	99.63 ± 0.47	98.81 99.92	0.998 ± 0.002	0.994 0.9996	0.049 ± 0.032	0.026 0.104

$P \leq 0.001$ for all correlations (r)
R^2 determination coefficient (%); r correlation coefficient; MDsd standard deviation of mean of individual differences (%); n number of participation (instrument – indirect method); MIR infrared (IR) technology with optical filters; MIR–FT IR spectroscopy with Fourier's transformations; UM ultrasonic method; BRB analysis by the blue and red box

III: *Expression of the relationship and the potential for calibration quality of indirect methods according to reference values (method) of milk protein (P)*

Instrument, method	n	R^2		r		MDsd	
		x ± sd	VR	x ± sd	VR	x ± sd	VR
MIR	7	98.56 ± 0.84	97.09 99.42	0.993 ± 0.004	0.9853 0.9971	0.035 ± 0.01	0.021 0.049
MIR–FT	10	98.72 ± 1.53	94.57 99.86	0.994 ± 0.008	0.9725 0.9993	0.033 ± 0.013	0.019 0.06
UM	4	83.66 ± 9.51	71.54 96.11	0.913 ± 0.052	0.8458 0.9804	0.127 ± 0.046	0.048 0.159
BRB	4	86.73 ± 7.42	75.26 93.33	0.93 ± 0.04	0.8675 0.9661	0.091 ± 0.03	0.063 0.137

$P \leq 0.001$ for all correlations (r)

IV: *Expression of the relationship and the potential for calibration quality of indirect methods according to reference values (method) of lactose (L)*

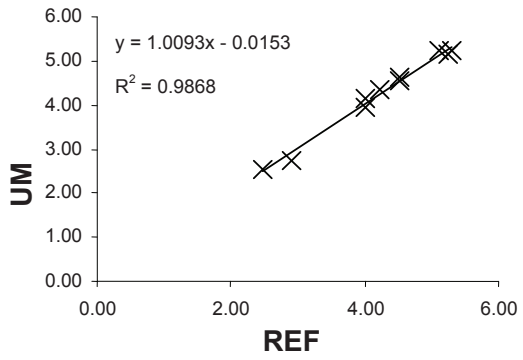
Instrument, method	n	R^2		r		MDsd	
		x ± sd	VR	x ± sd	VR	x ± sd	VR
MIR	7	95.57 ± 1.25	93.54 97.02	0.978 ± 0.006	0.9672 0.985	0.03 ± 0.004	0.025 0.037
MIR–FT	9	91.09 ± 8.97	65.96 95.75	0.953 ± 0.05	0.8122 0.9785	0.041 ± 0.02	0.029 0.097
UM	4	69.64 ± 12.79	52.42 88.53	0.831 ± 0.077	0.724 0.9409	0.083 ± 0.018	0.054 0.103
BRB	4	79.12 ± 13.38	61.77 92.85	0.886 ± 0.076	0.7859 0.9636	0.074 ± 0.022	0.051 0.106

$P \leq 0.001$ for all correlations (r)

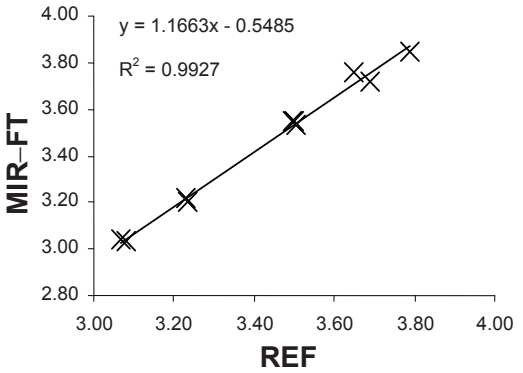
V: *Expression of the relationship and the potential for calibration quality of indirect methods according to reference values (method) of milk solids non fat (SNF)*

Instrument, method	n	R^2		r		MDsd	
		x ± sd	VR	x ± sd	VR	x ± sd	VR
MIR	4	99.12 ± 0.44	98.44 99.91	0.997 ± 0.003	0.9922 0.9995	0.029 ± 0.014	0.011 0.049
MIR–FT	10	99.26 ± 0.41	98.58 99.84	0.996 ± 0.002	0.9929 0.9992	0.043 ± 0.015	0.018 0.069
UM	4	91.05 ± 4.89	86.6 98.42	0.954 ± 0.026	0.9306 0.9921	0.103 ± 0.023	0.069 0.125
BRB	4	86.44 ± 6.47	78.31 95.78	0.929 ± 0.035	0.8849 0.979	0.14 ± 0.039	0.078 0.178

$P \leq 0.001$ for all correlations (r)



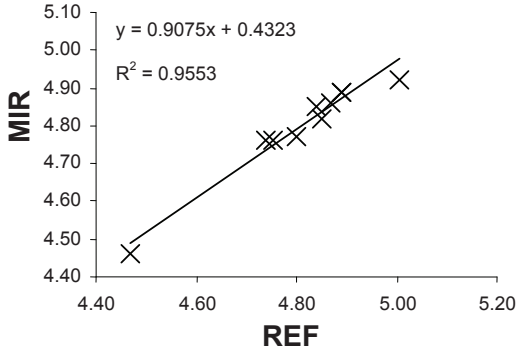
1: *The relationship between reference (REF) results (x; extraction and gravimetric method according to Röse–Gottlieb) and indirect method UM (y) for milk fat determination (F, %; the mean result)*
r = 0.993***; $P < 0.001$



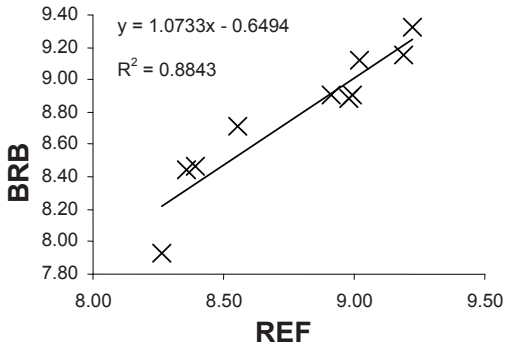
2: *The relationship between reference (REF) results (x; mineralization–destillation–titration method according to Kjeldahl) and indirect method MIR–FT (y) for milk crude protein determination (P, %; the mean result)*
r = 0.996***; $P < 0.001$

proportion of the variability in reference results than the UM and BRB methods (alternative): – for F it was 99.82 ± 0.25 and 99.9 ± 0.11 versus 98.78 ± 0.4 and 99.63 ± 0.47% (Tab. II); – for P it was 98.56 ± 0.84 and 98.72 ± 1.53 versus 83.66 ± 9.51 and 86.73 ± 7.42% (Tab. III); – for L it was 95.57 ± 1.25 and 91.09 ± 8.97 versus 69.64 ± 12.79 and 79.12 ± 13.38% (Tab. IV); – for SNF it was 99.12 ± 0.44 and 99.26 ± 0.41 versus 91.05 ± 4.89 and 86.44 ± 6.47% (Tab. V). Generally, regarding reliability, the results of conventional methods MIR and MIR–FT (Tabs. II–V) can be find as equivalent in terms of potential assurance of calibration quality.

Logically the similar relationships were between the methods (direct and indirect methods) and the individual components (F, P, L and SNF) also in correlation coefficients (Tabs. II–V). All the mentioned average values of r (x minus 1.64 × sd for confidence interval (CI, unilateral) at the 95% probability level; Grappin, 1987) in the tables can be used as standards for mentioned indirect analytical methods in dairying in quality evaluation of performed calibrations. In this case as a standard does not specify otherwise these r limits (in order MIR, MIR–FT, UM and BRB) could be: – for F 0.997,



3: *The relationship between reference (REF) results (x; polarimetric method) and indirect method MIR (y) for lactose determination (L, %; the mean result)*
r = 0.977***; $P < 0.001$

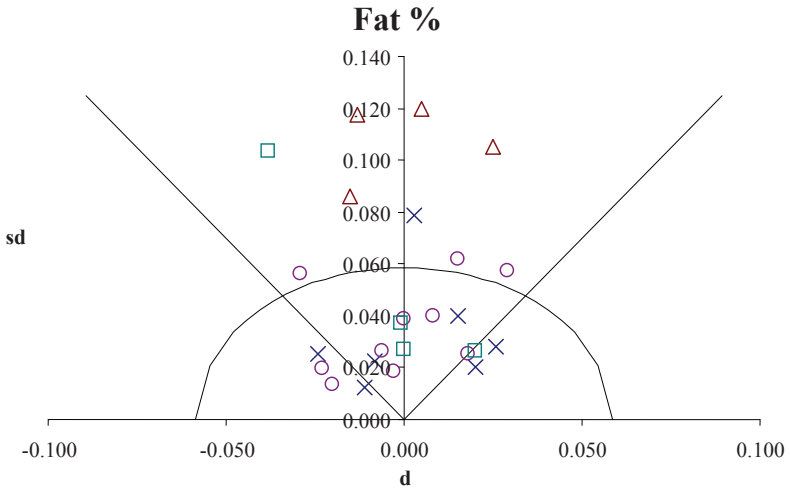


4: *The relationship between reference (REF) results (x; gravimetric method for total solids without F) and indirect method BRB (y) for determination of solids non fat in milk (SNF, %; the mean result)*
r = 0.94***; $P < 0.001$

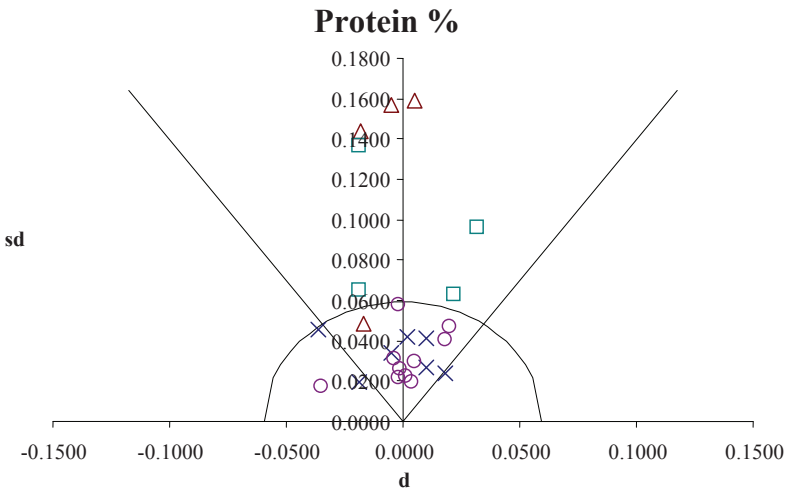
0.997, 0.99 and 0.995; – for P 0.986, 0.981, 0.828 and 0.864; – for L 0.968, 0.871, 0.705 and 0.761; – for SNF 0.992, 0.993, 0.911 and 0.872.

In connection with the foregoing, also the average standard deviations of mean of individual differences (MDsd) were generally lower for methods MIR and MIR–FT than UM and BRB at all the components of milk (F, P, L and SNF; Tab. II–V). Also these average values (MDsd) can be used as a standards (x plus 1.64 × sd for CI (unilateral) at the 95% probability level; Grappin, 1987) in evaluation of the quality of accepted calibrations of indirect methods. In this case as a standard does not specify otherwise these MDsd limits (in order MIR, MIR–FT, UM and BRB) could be: – for F 0.071, 0.068, 0.132 and 0.101%; – for P 0.051, 0.054, 0.202 and 0.14%; – for L 0.037, 0.074, 0.113 and 0.11%; – for SNF 0.052, 0.068, 0.141 and 0.204%.

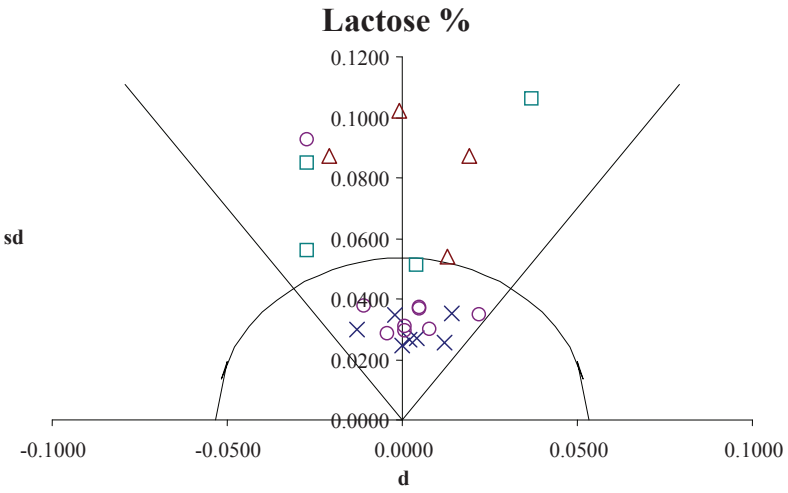
Today there are used the systems of real time milk composition measurement (AfiLab) in milking parlours. There is used the near infrared principle in a flow–through arrangement. The results are used for the management of the dairy herd. As reported by Katz (2007) and Ishay *et al.* (2011), the results may not be as accurate as in the laboratory. Nevertheless, their explanatory power for the animal increases just by regular measurement (real time application). For the specific severity of measurement conditions the determination values of relationships to calibration methods are lower for these procedures (64–76% for F, 45–52% for P and 19–52% for L; Karp and Petersson Wolfe, 2010) than the methods herein MIR, MIR–FT, UM and BRB



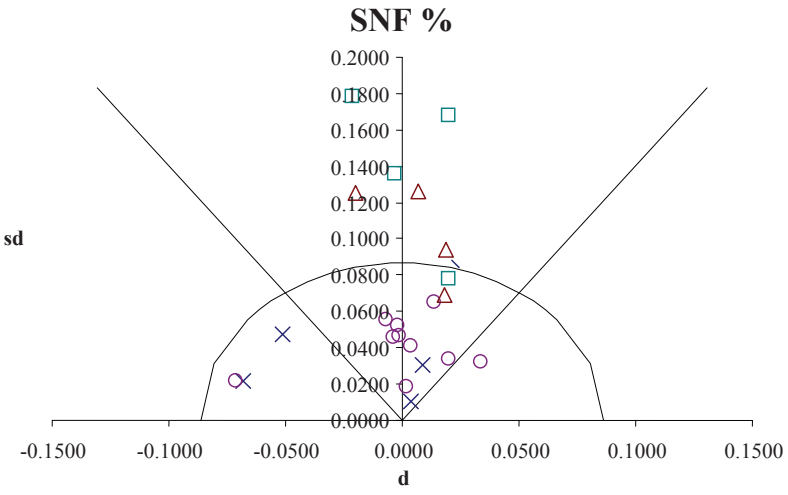
5: *The result of proficiency testing for milk fat determination using indirect methods after accepted instrument calibration (the validation of the F calibration)*
MIR cross; MIR–FT ring; UM triangle; BRB square; d = mean difference (indirect method – reference method value); sd = standard deviation of individual differences along samples; lines which go out from central position of graph represent statistical significance on level 5% (pair t–test, Student's distribution), points which are below lines are significantly different ($P \leq 0.05$), points over lines are insignificantly different ($P > 0.05$); bow incloses 90% of confidence interval of Euclidean distance from origin (calibration efficiency)



6: *The result of proficiency testing for milk crude protein determination using indirect methods after accepted instrument calibration (the validation of the P calibration)*



7: *The result of proficiency testing for lactose determination using indirect methods after accepted instrument calibration (the validation of the L calibration)*



8: *The result of proficiency testing for milk solids non fat determination using indirect methods after accepted instrument calibration (the validation of the SNF calibration)*

(on average ≥: 98.78 for F; 83.66 for P; 69.64% for L; Tab. II–IV). Furthermore, Perlín (2003) found good agreement with the SNF reference method results for UM method but methodologically he limited the acceptable consensus for the measurement of milk protein per range from 3.0 to 3.7%. This was practically fulfilled in this experiment solution (Tab. I).

From present point of view, with regard to the reference methods (alpha level, Grappin, 1993), MIR and FT–MIR can be classified as conventional methods (beta level) for official purposes such as the payment of milk according to quality and milk recording for genetic improvement and UM and BRB can be described as alternative methods for other purposes (level beta or gamma), such as advisory service in the dairying. In this sense it is analytically more efficient to calibrate UM and BRB directly according to results of reference methods (from alpha to beta similarly as at methods MIR and MIR–FT) than transfer the calibrations to UM and BRB from MIR and MIR–FT in the dairy laboratory system.

CONCLUSION

In the graphical evaluation of the calibration success of indirect methods using Euclidean distance (Ed in PT evaluation) from the origin (Figs. 5–8) can be seen vertical clustering of analytical methods (vertical variability – the degree of individual equality of method results, here more important indicator). Clusters of UM and BRB methods are usually above (longer Ed) clusters of MIR and MIR-FT methods in the stratification of graphs. This fact confirms the assessments performed above and clearly demonstrates the order of effectiveness of analytical methods. Despite this fact, it is also shown that the use of alternative methods (UM and BRB) may be advantageous for example for advisory purposes in the dairying, such as the control of diet of dairy cows and control of animal health and management to prevent the occurrence of production disorders (mastitis and ketosis) as mentioned in the introduction.

Furthermore, the above results can be used (after appropriate statistical transformation) as the standard limits in assessing the quality of calibrations (F, P, L and SNF) in dairy analysis system in the Czech Republic.

SUMMARY

The milk analyse result reliability is important for assurance of good health of animals and support of foodstuff chain quality. There are more direct and indirect methods for milk composition measurement (fat (F), protein (P), lactose (L) and solids non fat (SNF) content). Their number and expansion is growing up along increase of demands on animal health and milk product quality: infrared (IR) spectrometry in mid (MIR and MIR–FT) and near (NIR) IR range (for F, P, L, SNF); photocolorimetry (for P); ultrasonic method (F, P, L, SNF); nephelometry (F). The goal of this study was to evaluate some reference and routine milk analytical procedures and carry out mutual comparison of their results. Today, the above mentioned various methods are used in milk laboratories on univerisities, dairy plants, agricultural enterprises and in milk recording. Therefore, information about comparison of result reliability is important for laboratory staff. The direct reference analyses carried out including abbreviations and units of measurement were as follows: F, fat content (according to Röse–Gottlieb method; %); P, crude protein content (according to Kjeldahl method; %); L, lactose (monohydrate, %; by polarimetric method); SNF, solids non fat (%; by gravimetric method). F, P, L and SNF were determined also by various indirect methods: – MIR (infrared (IR) technology with optical filters in 4 laboratories; – MIR–FT (IR spectroscopy and use of Fourier's transformations) in 6 laboratories; – ultrasonic method (UM) in 1 laboratory; – analysis by the blue and red box (BRB) in 1 laboratory. There were used 10 calibration (reference) milk samples prepared by reference methods. Difference statistic and linear regression was performed. The closeness of result relationships of compared methods is important for evaluation. Shift on the axis can be easily corrected. Therefore, coefficient of determination (R^2), correlation coefficient (r) and standard deviation of the mean of individual differences (MDsd, for n) were evaluated. These are suitable to objective assess the quality of the calibration. All obtained correlation coefficients (r; for all indirect and alternative methods and all components of milk) were statistically significant ($P \leq 0.001$). It is evident that the methods MIR and MIR–FT (conventional) explained considerably higher proportion of the variability in reference results than the UM and BRB methods (alternative): – for F it was 99.82 ± 0.25 and 99.9 ± 0.11 versus 98.78 ± 0.4 and 99.63 ± 0.47%; – for P it was 98.56 ± 0.84 and 98.72 ± 1.53 versus 83.66 ± 9.51 and 86.73 ± 7.42%; – for L it was 95.57 ± 1.25 and 91.09 ± 8.97 versus 69.64 ± 12.79 and 79.12 ± 13.38%; – for SNF it was 99.12 ± 0.44 and 99.26 ± 0.41 versus 91.05 ± 4.89 and 86.44 ± 6.47%. Regarding reliability, the results of conventional methods MIR and MIR–FT can be find as equivalent in terms of potential assurance of calibration quality. All r average values (x minus 1.64 × sd for confidence interval (CI, unilateral) at the 95% probability level) can be used as standards for indirect analytical methods in calibration quality evaluation (in order MIR, MIR–FT, UM and BRB): – for F 0.997, 0.997, 0.99 and 0.995; – for P 0.986, 0.981, 0.828 and 0.864; – for L 0.968, 0.871, 0.705 and 0.761; – for SNF 0.992, 0.993, 0.911 and 0.872. Also MDsd average values

can be used as a standards (x plus 1.64 × sd) in evaluation of calibration quality of indirect methods: – for F 0.071, 0.068, 0.132 and 0.101%; – for P 0.051, 0.054, 0.202 and 0.14%; – for L 0.037, 0.074, 0.113 and 0.11%; – for SNF 0.052, 0.068, 0.141 and 0.204%.

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