Relationships between the results of various methods of urea analysis in native and enriched milk

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ABSTRACT: Milk urea concentration (MUC) is a suitable indicator of the health and nutrition state of dairy cows. MUC is in relation to their reproduction performance, longevity and technological milk indicators. The interpretation correctness of results depends on their reliability. There are a lot of principles of MUC analyses. Their results can be affected by a number of interferential factors. Disproportions were noticed in practice. Therefore the sources of variation in results are studied. The goal of this study was to investigate relationships between different methods of MUC determination with the use of standard samples of native milk with an artificial urea addition. After evaluation I (n = 7) the results of methods BI-1 and BI-2 (photometrical ones with diacetylmonoxime) were disqualified because of poor recovery (R), poor correlation (C) with other methods, higher random error (RER) and highest systematic error (SE). Evaluation II is more effective with stricter discrimination limits. Cs of all methods mutually (0.977 up to 0.998; $P < 0.001$) confirmed the methods as effective with the exception of BI-2 with poor Cs (0.713 up to 0.774), poor R (16.0 up to 69.0%) and high RER ±5.292 mg/100 ml. R of better methods was 44.0 up to 96.7%. The BI-1 method had good Cs (0.986 up to 0.994; $P < 0.001$), higher SE –7.546 mg/100 ml and poorer R (48.5 up to 75.3%). BI-1 method was a case of mistaken performance. BI method could be improved by the use of more samples in calibration. FT-MIR method (infra-analysis) has good addition R 69.5 up to 95.0% and Cs 0.981 up to 0.994 ($P < 0.001$). EH method (photometrical one with Ehrlich's agent) has good R 59.0 up to 96.7%, higher SE 4.755 (I) and 2.556 (II) mg/100 ml and close Cs 0.977 up to 0.994 ($P < 0.001$). UR method (ureolytical difference-conductometric) showed the best combination of results about R, C, SE and RER. MUC measurement was almost independent of fat in milk (r = 0.16 for UR and 0.01 for FT-MIR; $P > 0.05$) and MUC of both the methods did not increase significantly with lactose increase (r = 0.16 and 0.27; $P > 0.05$), which increased logically (r = –0.88; $P < 0.001$) during the fat concentration increase. The relationship of MUC results between UR and FT-MIR was significant (validation r = 0.96; $P < 0.001$) at average difference –0.93 ± 1.663 mg/100 ml. It is possible to see the result reliability as good after calibration performance of FT-MIR according to results of UR. It is not necessary to see the effects of fat, protein and lactose on MUC methods as substantial. FT-MIR method for MUC has good result reliability at the use of native milk samples, incidentally with urea additions. It is suitable to calibrate the FT-MIR method according to specific determination of MUC (UR). However, the most important for elimination of disproportions is the calibration method with concrete audited R, though nonspecific.

Keywords: cow; milk; urea; analytical methods; result reliability; recovery; correlation

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Urea is a final product of protein metabolism in the organism and the knowledge of the levels of excreted urea is one of the important indicators of a correctly formulated feed ration. Besides the liver, urea as a final metabolite is processed also in the kidneys. After liver synthesis urea is transported into kidneys and excreted in urine by kidneys. Urea enters from the blood into other body fluids and into milk as well. The factors influencing the urea concentration in the organism and milk are as follows:

– the input of protein and energy (generally, the higher content of crude protein in the feed ration leads to the higher concentration of urea in milk and higher input of energy often decreases the milk urea concentration (MUC));
– the input of protein digestible in the rumen and of protein non-digestible in the rumen;
– the input of water and dry matter in the feed ration (dehydration is expected to increase the urea concentration);
– the health state, especially the functional state of liver and kidneys;
– some diseases;
– pasture (higher urea content by pasture);
– the time of milk sampling after feeding (Gustafsson and Palmquist, 1993; Carlsson and Bergström, 1994).

For this reason the determination of MUC was introduced as a control of nitrogen-energy metabolism of cows (Erbersdobler et al., 1979; Oltner and Wiktorsson, 1983; Kirchgessner et al., 1985; Homolka and Vencl, 1993; Jonker et al., 1998; Strusińska et al., 2006; Zhai et al., 2006), i.e. the nutrition level as a means for the prevention of health problems in a number of practical systems. Many impacts of the nutrition state were described including milk production and other non-nutritional management factors like lactation characteristics (Hanuš et al., 1993; Jílek et al., 2006).

Since the times of basic comparisons, promoted in Poland (Michalak, 1972; Michalak et al., 1978), the existence of relatively expressive disproportions in absolute numbers between the laboratories in analyses of different milk components has been well known, which can result from many technical reasons like different or false milk sampling, different milk sample treatments, different principles and methods of measurements, local or breed specificity of calibration of measurement instruments, technical defects of the instruments, different attitude of the staff, etc. Due to practical aspects of the above-mentioned occurrence of differences in the results of milk analyses between laboratories, which is often criticized by users of the results, many authors have analysed and explained the existing variability of the results and differences in the values in comprehensive studies (Sherbon, 1975; Biggs, 1978; Vines et al., 1986; Coleman and Moss, 1989; Barbano et al., 1991; Golc-Teger et al., 1996; Golc-Teger, 1996, 1997). For this reason the plan of so called "netting of laboratories" was performed, where the check of variability of the results using reference milk and other standards (defined samples) and methods of proficiency testing (ring or star tests) is possible. This principle comprises the specification of different levels of work nets and their hierarchy and design of statistical evaluation of the results, like the method of determination of referential values, evaluation of the variability of errors, etc. (Grappin, 1987, 1993; Leray, 1993; Wood, 1994; Wood et al., 1998). For the sophisticated value of this access it became a part of the AQA (analytical quality assurance) systems of milk laboratories as well as their accreditation processes and audits. Different basic accesses and processes were published (Arndt et al., 1991; Leray, 1993; Heeschen et al., 1994; Wood, 1994; Pitkälä et al., 2005).

The disproportions in analytic results of accredited laboratories are mostly defined by a failure in the proficiency test of analytical capability or by the uncovering of the areas of uncertainty by the comparison of the inter-laboratory results of the identical samples, of milk in this case. The higher occurrence of the result disproportions or errors decreases the reliability of the analytic results. The result disproportions have theoretically two sources, a systematic and an accidental error. The statistical evaluation of data by the comparison of analytical results, which describes current and general reliability of the results, many times has a design which makes possible an eventual diagnosis of the type of error in a concrete laboratory. These systems usually comprise different standardised numerical and relevant graphical expressions as the tests of remoteness (mostly Grubbs's), Z-score, Euclidian distance, Youden plot, regulation Shewhart diagrams and their mutual combinations. The above-mentioned approaches were described in several papers that mostly came out recently (Grappin, 1987; Arndt et al., 1991; Leray, 1993; Heeschen et al., 1994; Wood, 1994).

The area of the analysis of nitrogen compounds in milk is a relatively comprehensive theme with a

number of features and methodological conventions which can lead to comparative problems (Grappin and Lefier, 1993; Hanuš et al., 1995). Evidently, it is known that many methodical and analytical disproportions have occurred. This is true especially of urea as a minority milk component. There exist many different applied analytical principles of MUC determination (Oltner and Sjaunja, 1982; Rajamäki and Rauramaa, 1984; Carlsson and Bergström, 1994; Hanuš et al., 1997, 2001; Lefier, 1999; Broutin, 2000, 2006a,b; Peterson et al., 2004). Their number is much higher than in the other especially majority milk compounds. They can lead to different results under certain conditions. Nevertheless, the reliability of the results is a crucial factor for their relevant interpretation and consequent prevention of problems with nutrition and health disorders in cows. The theme has been in the centre of attention for a longer period (Herre, 1998; Klopčič et al., 1999; Peterson et al., 2004). In the Czech Republic many disproportions in results and consequently also in their interpretation occurred during the gradual merging of laboratories (2004–2006). Despite this fact, the relevant emphasis on consistent control by the proficiency testing of analytical reliability of the results was rather missing for many organisation reasons. One of the reasons was the undesirable multiplicity and many commercial influences on the milk testing in the Czech Republic. The favourable effects of the objective independent proficiency testing in a laboratory network and its evaluation were reported already before (Grappin, 1993; Leray, 1993; Wood, 1994; Wood et al., 1998; Hanuš et al., 2000).

The goal of this study was to investigate the relationships between different recently applied principles of the methods of MUC determination in the Czech Republic, especially between new modern methods (measurement in the mid-area of infrared spectrum with the application of Fourier's transformations, FT-MIR) using the control samples of native milk and with addition of urea, and to derive the relevant methodical recommendations from the theoretical relationships for improvement of reliability and practical interpretation of produced analytical results.

MATERIAL AND METHODS

Analytical methods

More detailed characterization of the methods of MUC determination can be found in preceding pa-

Table 1. Characterization of the used methods of MUC analyses

Abbreviation	Name	Principle of reaction	Principle of measurement	Used calibration	Specificity	Occurrence in test
UR	Ureakvant	ureolysis of urea	difference in conductivity before and after reaction	five-level calibration line according to standards with given artificial addition of urea (milk standards)	yes	3×
BI	BioLaTest	dying of urea by diacetylmonoxime	photometrically (525 nm)	line-slope according to calibration standard with given urea content (non-milk standard)	no	2×
EH	Ehrlich	dying of urea by para-dimethylaminobenzaldehyde	photometrically (420 nm)	five-level calibration line according to standards with given artificial addition of urea (non-milk standards)	no	1×
FT-MIR	FT infra-analysis Foss 6000	IR absorption of urea in whole spectrum (interferometer)	manipulation of recovery of signal by Fourier's transformations	multilevel linear regression to results of selected reference method (milk standards)	no	1×

specificity = utilisation of hydrolytic reaction by means of urease (enzymatic method)

pers (Hanuš et al., 1997, 2001; Klopčič et al., 1999). A short description is in Table 1. The colorimetric methods were performed with Spekol 11 (Carl Zeiss, Jena, Germany) equipment. The results of the milk components (content of fat (F), crude proteins (CP), lactose monohydrate (L)) were performed with Bentley 2000 (Bentley Instruments, USA) equipment, which was calibrated once a month according to the results of reference methods (Gerber's, Kjeldahl's and polarimetric method).

Experiment 1

Ten samples of native raw milk were taken on chosen farms of Holstein and Czech Pied breeds and used in an experiment. The samples were immediately transported to chosen laboratories in cold state. The MUC analyses (seven participants) were carried out in 6 laboratories (nearly all of them with accreditation certificate and two of them with the status of National Reference Laboratory for Raw Milk) in the Czech Republic using 4 analytical methods.

The artificial methodical enhancement of urea concentration in several selected samples of native milk (No. 6, 8, 9, 10) in a set of samples in proficiency test was achieved by the application of additions with higher and high urea concentration in a milk matrix solution so that the resulting concentration of urea in these samples would be by 10 (2×), 20 (1×) and 30 (1×) mg/100 ml higher as compared to basic concentrations in original samples (No. 4, 7, 2 and 7). These additions served for the calculation of recovery of used analytical methods for the analysis of reliability of the MUC results. Then addition (difference) recovery was determined as a difference in the values which were measured before and after the application of additions and 100% was the value of the weight of the addition.

Evaluation of results

The results (Table 2) were evaluated by the calculation of recovery and determination of Euclidian distance for reliability of laboratory results. The

Table 2. The values of MUC (mg/100 ml) measured by the particular analytical methods

Method sample	FT-MIR	UR-1	UR-2	EH	BI-1	BI-2	UR-3	REF. I	REF. II
1	19.1	26.1	27.8	27.1	17.4	30.4	26.5	24.92	26.88
2	25.4*	30.1	28.9	30.0	21.2*	30.7	29.7	29.88	29.68
3	19.7	26.0	25.9	27.0	18.0	33.7	26.3	25.23	26.30
4	21.2	28.8	27.0	28.5	19.8	22.6	28.0	25.13	28.08
5	26.2	27.7	29.7	31.8	21.8	27.0	30.6	27.37	29.20
6	29.3	33.2	34.0	37.0	25.3	25.4	35.1	31.34	33.72
7	24.8	27.8	30.2	35.1	20.2	24.0	30.7	27.54	29.72
8	33.3	35.0	36.7	41.0	26.6	28.2	37.4	34.03	36.68
9	39.3	41.7	43.7	45.5	30.9	33.9	44.1	39.88	42.86
10	53.3	56.7	58.7	64.1	42.8	44.7	59.3	54.23	58.42
$\bar{x}$	29.2	33.3	34.3	36.7	24.4	30.1	34.8	31.96	34.15
SD	10.0	9.0	9.6	10.8	7.3	6.1	9.7	8.7	9.4
CV (%)	34.3	27.2	28.0	29.5	29.9	20.2	27.9	27.1	27.6
Min	19.1	26.0	25.9	27.0	17.4	22.6	26.3	24.9	26.3
Max	53.3	56.7	58.7	64.1	42.8	44.7	59.3	54.2	58.4
R max–min	34.2	30.7	32.8	37.1	25.4	22.1	33.0	29.3	32.1

FT-MIR = infrared method in the mid-area of the spectrum with Fourier's transformations; UR-1, UR-2 and UR-3 = specific, difference-conductometric method Ureakvant; EH = photometric method with Ehrlich solution; BI-1 and BI-2 = photometric method BioLaTest; REF. I and REF. II = reference values after the statistical exclusion of remote results (*Grubbs' test on the significance level 95%) from the total set (I) and after the exclusion of remote results of methods (II); n = number of cases; $\bar{x}$ = arithmetical mean; SD = standard deviation; *CV* = coefficient of variation in (%); min = minimum; max = maximum

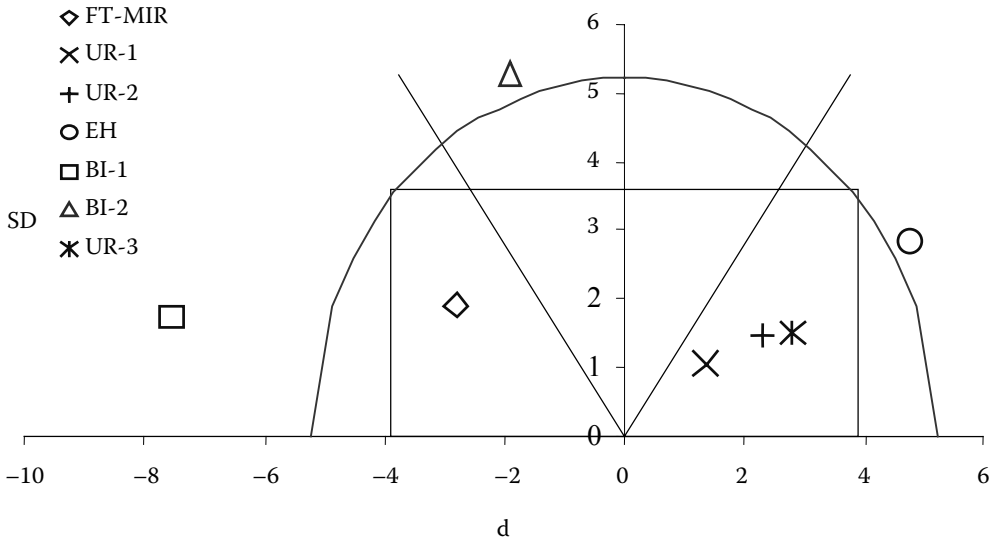

Figure 1. The Euclidian distance of participants (I; n = 7) in determination of urea concentration in milk (MUC) from origin (RE)

Table 3. General ordering of participants (I; n = 7) in MUC determination according to Euclidian distance from the origin (RE)

LAB	d	SD	RE	t	P
UR-1	1.355	1.055	1.7173	4.06	**
UR-2	2.305	1.461	2.7290	4.99	***
UR-3	2.815	1.486	3.1831	5.99	***
FT-MIR	–2.795	1.914	3.3875	4.62	**
EH	4.755	2.821	5.5288	5.33	***
BI-2	–1.890	5.292	5.6194	1.13	NS
BI-1	–7.546	1.731	7.7420	13.78	***

LAB = laboratory (method, participant); d = average difference (mg/100 ml); SD = variability of average difference (standard deviation); RE = Euclidian distance; t = value of t testing criterion of pair test; P = statistical significance (NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$)

principles of these procedures were described in detail in preceding papers (Leray, 1993; Hanuš et al., 1998). Standard deviations and variability of individual values of these deviations in participants (laboratories, methods) from reference values and Euclidian distance (RE) of the laboratories from the centre (origin) were calculated. The correlation coefficients between combinations of participants were also calculated. In tables and figures it is possible to find the positions of the laboratories and methods regarding the reliability of the measured results. The table and graphic discrimination limits of the success of participation in the test were derived in the following way:

Semicircle:

(1) for the average difference (d) as 1.96-times multiplied value of standard deviation of d of the set, i.e. at the conventional level 95% of confidence interval
(2) for standard deviation of the average difference (SD) by the method of robust estimate as the sum of medians of sd set and 1.65-times multiplied standardized quartile estimate of standard deviation, i.e. at the conventional level of 95%
(3) by the combination of both previous (d and SD) limits on the basis of the mean the limit was created (semicircle in the graph) with discrimination ca. 10%

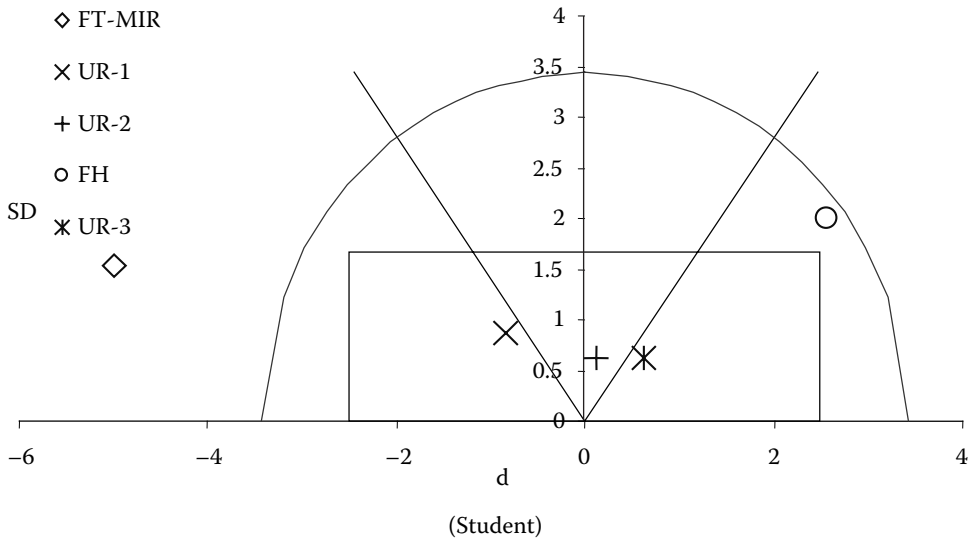

Figure 2. The Euclidian distance of participants (II; n = 5) in determination of urea concentration in milk (MUC) from origin (RE)

Table 4. General ordering of participants (II; n = 5) in MUC determination according to Euclidian distance from the origin (RE)

LAB	d	SD	RE	t	P
UR-2	0.106	0.633	0.6418	0.53	NS
UR-3	0.616	0.633	0.8833	3.08	*
UR-1	–0.844	0.872	1.2136	3.06	*
EH	2.556	1.996	3.2430	4.05	**
FT-MIR	–4.994	1.530	5.2231	10.32	***

For expanations see Table 3

Tetragon:
(4) for d as the value of one standard deviation of d set
(5) for SD as the sum of the means of the SD set and its standard deviation

Experiment 2

The goal of this experiment was the comparison of the MUC measurement by the method of infrared analysis and by the ureolytical method in milk samples with different contents of milk components. The different fat content was achieved by the sampling of milk in three breeds of cattle. Raw samples were collected from the breed Jersey (J100), Czech Pied (C100) and Holstein (H100). The collected milk (samples No. 1, 2, 3) was stored in cold so that the different fat fractions of the milk could be obtained from each sample. Further samples were collected from the top (No. 4, 7, 10), middle (No. 5, 8, 11) and bottom part (No. 6, 9, 12) of the storage vessel. The collection of 12 milk samples with extreme differences in milk components, especially in the case of fat, was prepared in this way. The results were evaluated by means of basic statistical methods and linear regression method.

RESULTS AND DISCUSSION

Experiment 1

The evaluation of the results (I and II; Table 2) by the RE method was done twice in complete arrangement (Figure 1; Table 3) and then with five participants only, while the other two were excluded due to the weakest recovery. Moreover, the first also due to weak correlations (Table 5) with other methods and higher value of accidental error (SD)

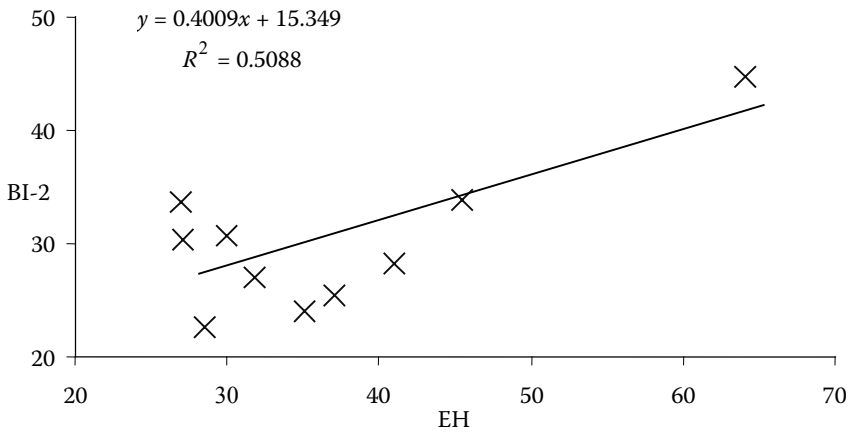

Figure 3. The freest linear regression relationship (n = 10; r = 0.713; P < 0.01) between the methods of MUC determination (mg/100 ml), EH × BI-2

Table 5. Correlation coefficients of linear regression of the relationships between methods of MUC determination

	FT-MIR	UR-1	UR-2	EH	BI-1	BI-2
UR-1	0.981					
UR-2	0.989	0.990				
EH	0.988	0.977	0.991			
BI-1	0.994	0.992	0.991	0.986		
BI-2	0.723	0.774	0.767	0.713	0.736	
UR-3	0.994	0.992	0.998	0.994	0.997	0.747

and the second due to the highest value d, which can be interpreted as a more expressive systematic error (Figure 1; Table 3). The second evaluation of II (Figure 2; Table 4) is due to this more effective regarding the fact that the reliability of results was evaluated by the RE method. Logically it is possible to observe expressively lower (stricter) discrimination limits and higher relevancy of the test in terms of demands on participants. In both the cases the BI method was excluded. The correlations of nearly all the participants (Table 5; from 0.977 to 0.998; P < 0.001) are very narrow and confirm the used methods as the effective ones with the exception of one case of BI method with very poor correlations (from 0.713 to 0.774) as compared to the other participants, which was confirmed by the week recovery (Figure 5; from 16.0 to 69.0%) and high SD (Figure 1; Table 3; accidental error ± 5.292 mg/100 ml). The recovery of more successful methods was from 44.0 to 96.7% (Figure 5; Table 6). Due to the fact that the second method BI-1 had good correlations (from 0.986 to 0.994; P < 0.001; Table 5) and higher negative d (systematic error –7.546 mg/100 ml; Table 3), which was confirmed by mostly weaker recovery (from 48.5 to 75.3%; Table 6), it is possible to

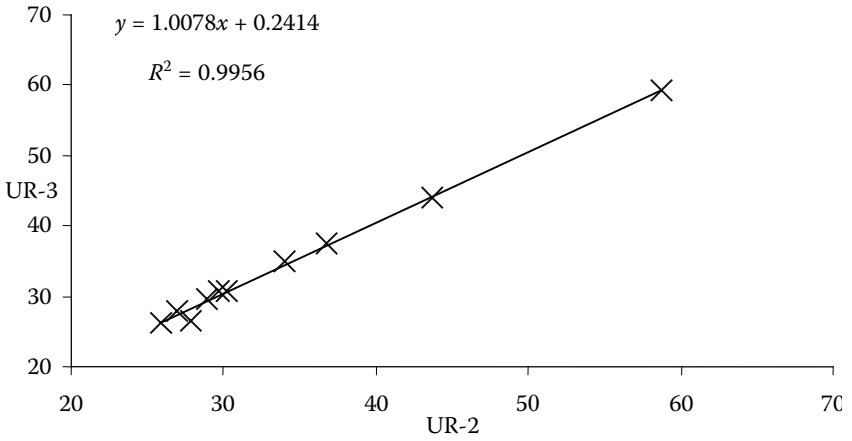

Figure 4. The closest linear regression relationship (n = 10; r = 0.998; P < 0.001) between the methods of MUC determination (mg per 100 ml), UR-2 × UR-3

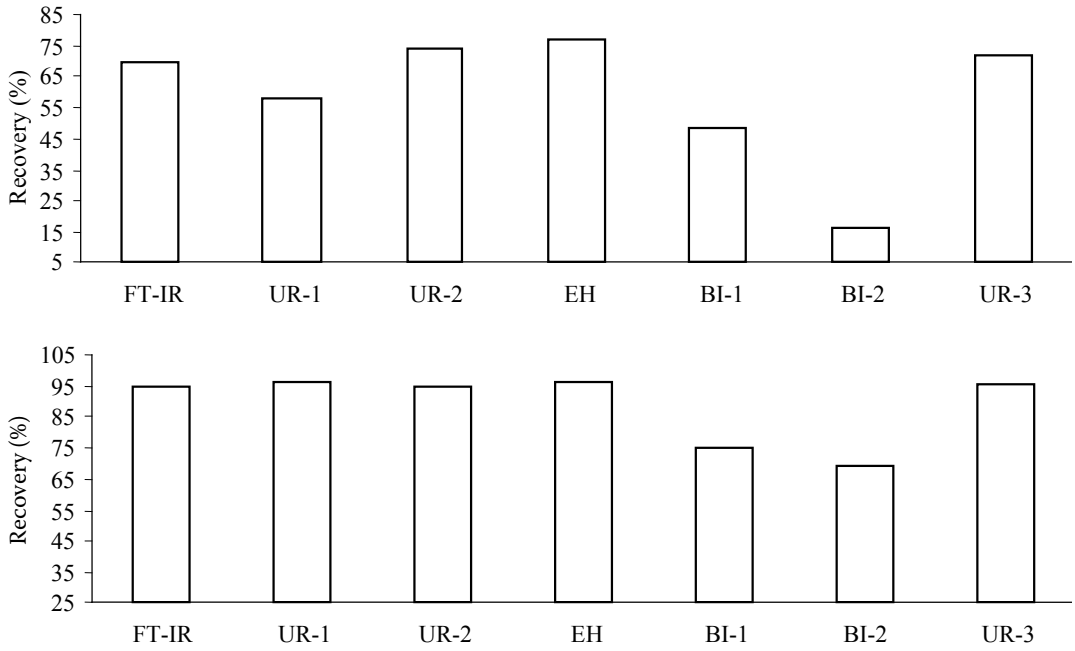

Figure 5. Recovery (R in %) of analytical methods of MUC determination in samples (No. 2 and 7) with addition of 20 (upper graph) and 30 (lower graph) mg/100 ml

Table 6. The values of addition recovery (R) for MUC by four samples of native milk with artificial additions

Addition method	10 mg/100 ml		10 mg/100 ml		20 mg/100 ml		30 mg/100 ml	
	d	(%)	d	(%)	d	(%)	d	(%)
FT-MIR	1.9	81.0	1.5	85.0	6.1	69.5	1.5	95.0
UR-1	5.6	44.0	2.8	72.0	8.4	58.0	1.1	96.3
UR-2	3.0	70.0	3.5	65.0	5.2	74.0	1.5	95.0
EH	1.5	85.0	4.1	59.0	4.5	77.5	1.0	96.7
BI-1	4.5	55.0	3.5	65.0	1.3	48.5	7.4	75.3
BI-2	7.1	29.0	5.8	42.0	16.8	16.0	9.3	69.0
UR-3	2.9	71.0	3.3	67.0	5.6	72.0	1.4	95.3

R = recovery in (%); d = difference R from 100% = additive weight of urea

conclude that the first case was not a question of the method correctness but of its local false application probably. The second case shows that the method can work correctly under the presumption of more real calibration. Here the object of improvement can be the fact of the methodical use of one calibration sample solely. The use of more samples in the calibration would remove the strong accidental effect of incidental error of one standard, which becomes a systematic error for all the series of samples belonging to the relevant calibration and a source of accidental error for the method generally in time.

The method FT-MIR as a main subject of interest in evaluation revealed surprisingly good addition recovery (from 69.5 to 95.0%; Table 6; Figure 5) and reliability of the results compared to the other methods (correlations from 0.981 to 0.994; $P < 0.001$; Table 5) and in comparison with former MIR methods on the basis of optical filter technology (Herre, 1998; Hanuš et al., 2001). Neither was the systematic error d for FT-MIR in the first evaluation (I) more expressive –2.795 mg/100 ml, in the second (II) case –4.994 mg/100 ml, which at the common variability (accidental error, SD) can be corrected by calibration (Tables 3 and 4;

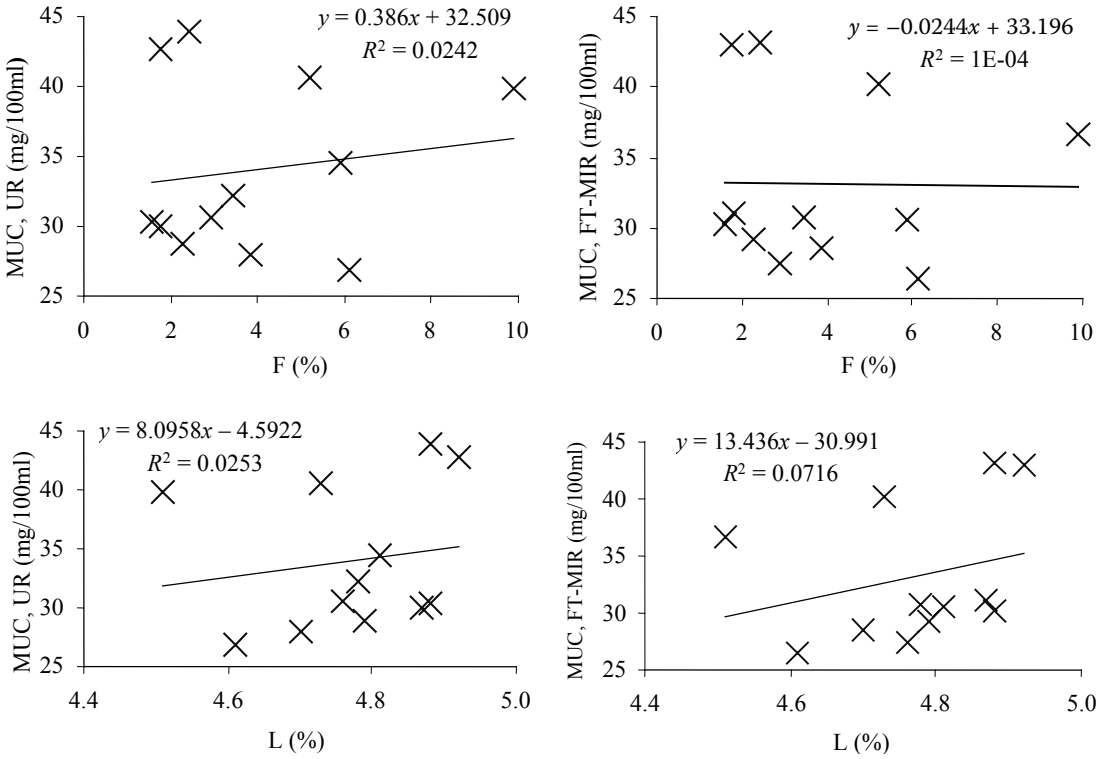

Figure 6. Linear regression relationships between F and L and the results of UR and FT-MIR methods of MUC determination

Figures 1 and 2). For instance, Peterson et al. (2004) also found recovery just 47.1 ± 9.9% for Foss 4000 system (MIR, filters) while it was 95.4 ± 10.1% for Foss 6000 (FT-MIR).

The EH method revealed the relatively very good values of addition recovery (from 59.0 to 96.7%; Table 6; Figure 5) although it has a slightly higher positive systematic error in both the evaluations 4.755 and 2.556 mg/100 ml also with the higher value of accidental error SD (Figures 1 and 2; Tables 3 and 4). The correlations with other methods were also tight (from 0.977 to 0.994; $P < 0.001$; Table 5; Figure 3).

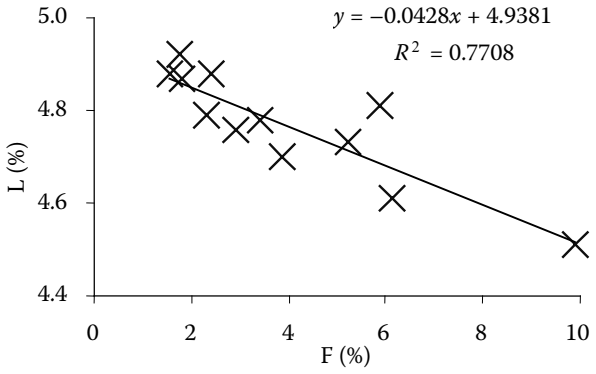

Figure 7. Linear regression relationships between fat and lactose content in control samples

Nevertheless, the EH method did not reach the parameters of result reliability of the specific UR method, which showed the best combinations of the results in recovery, correlation, systematic and accidental error (Figures 1, 2, 4 and 5; Tables 3, 4, 5 and 6) in two out of three cases (the worse one is probably the local case). This reflects the previous positive evaluations (Klopčič et al., 1999; Hanuš et al., 2001). Similarly, the good parameters of reliability of the results of the MUC analysis were also reported by Lefier (1999; in the framework of investigations of IDF – International Dairy Federation) for specific methods (with urea), manual one and automatic one with photometric and pH differential output of measurement, AFNOR (V04-217, reference method) and Eurochem.

The results show the generally good effectivity regarding the reliability of the results of the methods UR, EH and FT-MIR and thus the possibility to give information for relevant practical interpretation in the area of the control of the nutrition and health state of cows. The higher systematic methodical support of the reliability assurance of the results

Table 7. The results of MUC of two analytic methods (UR and FT-MIR, direct and indirect, calibrated and uncalibrated) in milk samples with modified fat content

Breed	Fraction	F	CP	L	MUC FT-MIR	MUC UR	MUC DIF
C	original	3.43	3.43	4.78	30.7	32.2	–1.5
C	high	5.89	3.36	4.81	30.6	34.5	–3.9
C	middle	1.79	3.38	4.87	31.1	30.0	1.1
C	bottom	1.58	3.39	4.88	30.2	30.3	–0.1
H	original	3.86	3.46	4.70	28.5	27.9	0.6
H	high	6.11	3.41	4.61	26.4	26.9	–0.5
H	middle	2.91	3.50	4.76	27.5	30.6	–3.1
H	bottom	2.29	3.52	4.79	29.2	28.8	0.4
J	original	5.22	3.93	4.73	40.2	40.6	–0.4
J	high	9.88	3.75	4.51	36.6	39.9	–3.3
J	middle	2.41	4.05	4.88	43.2	43.9	–0.7
J	bottom	1.76	4.08	4.92	43.0	42.7	0.3
$\bar{x}$		3.93	3.61	4.77	33.10	34.03	–0.93
SD		2.464	0.272	0.120	6.028	6.116	1.663
$\bar{x}$	original	4.17	3.61	4.74	33.13	33.57	–0.43
$\bar{x}$	high	7.29	3.51	4.64	31.20	33.77	–2.57
$\bar{x}$	middle	2.37	3.64	4.84	33.93	34.83	–0.90
$\bar{x}$	bottom	1.88	3.66	4.86	34.13	33.93	0.20

MUC = concentration of urea in milk (mg/100 ml); F = fat (%); CP = crude proteins (%); L = lactose (%); DIF = difference in MUC (FT-MIR – UR) in mg/100 ml; C = Czech Pied, H = Holstein, J = Jersey; $\bar{x}$ = arithmetical mean; SD = standard deviation

would be beneficial in the case of BI method (current variant), minimally by the enhancement of the calibration points.

Experiment 2

The results of MUC analyses using two methods (UR and FT-MIR) in samples of three breeds, according to the modified F in identical samples and consequently the other components (CP and L), are shown in Table 7. The possible effects of major components on the results of MUC measurements are shown by the linear regression model in Figure 6. The MUC measurement was dependent on F in milk to a certain extent in the case of UR method (r = 0.16 for UR and 0.01 for FT-MIR; $P > 0.05$) and MUC insignificantly increased along the enhancement of L (r = 0.16 and 0.27; $P > 0.05$), which logically significantly degreased (r = –0.88; $P < 0.001$; Figure 7) due to the enhancement of the F concentration. The mentioned comparison is usable for interpretation because F was strongly manipulated and L was relatively constant in all original samples. Carlsson and Bergström (1994)

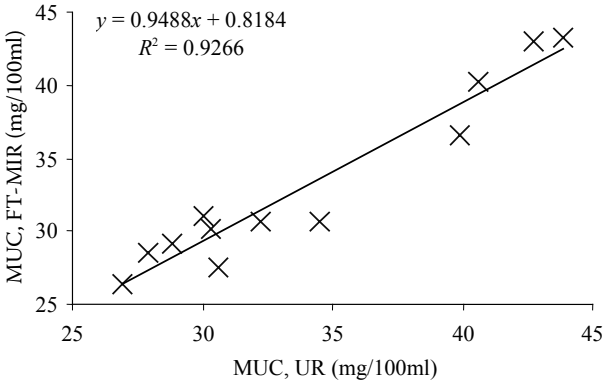

Figure 8. Linear regression relationships between MUC determined by two methods, direct and indirect, UR and FT-MIR, as a validation relationship

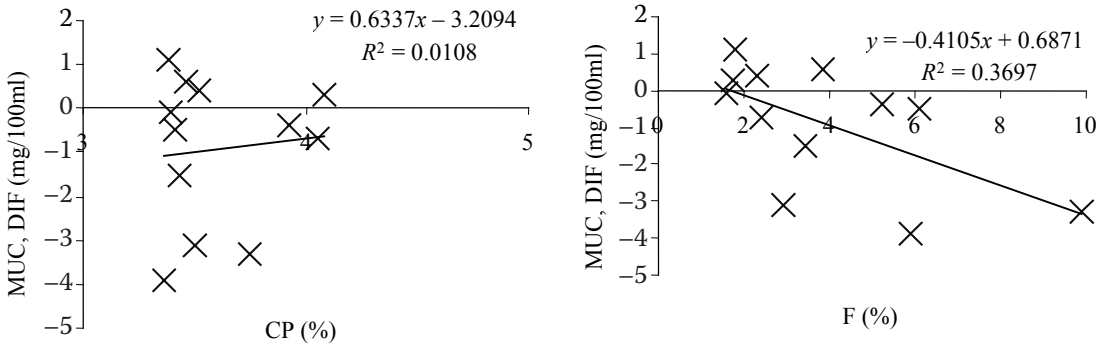

Figure 9. Linear regression relationships between CP and DIF MUC and F and DIF (FT-MIR – UR)

did not found any effect on the differences in MUC between the beginning and the end of milking (different F) by FIA method when the MUC was calculated in the water phase of milk. Neither did the MUC change significantly after storage during ten days at 4°C without sample preservation. Using the bronopol preservation it did not change after 17 days and the freezing point did not influence MUC. Here the important influence of CP (r = 0.90 and 0.94; $P < 0.001$) already originated from concentrations of both components in original samples and thus did not have an explicatory capability for this attempt of evaluation of the reliability of the MUC results. The relationship of the MUC results between methods (UR and FT-MIR) was significant (validation $r = 0.96$; $P < 0.001$; Figure 8) by the average difference (FT-MIR – UR) –0.93 and its variability ±1.663 mg per 100 ml (Table 7). It is possible to interpret these results, like the whole validation relationship (certain delay after calibration performance of FT-MIR according to the UR results), as a very good result regarding the evaluated reliability. The evaluation of the effects of major components (F, CP and L) on differences (DIF) in the MUC results between methods (FT-MIR – UR; Figure 9) shows an important relationship of F with higher MUC at higher F for the UR method, which confirms the previous conclusion (Figure 7). In the cases of proteins and lactose the relationships were not significant ($P > 0.05$; Figure 9). Nevertheless, in general it is not necessary to consider the effects of major milk components as substantial for both methods of MUC determination in the construction of methodical procedures in the practice of milk laboratories.

The artificial methodical enhancement of urea concentration in several selected standard samples of native milk in a set of samples in proficiency testing was achieved by application of the addition of a high concentration of urea into the solution of milk matrix so as the resulting concentration in a new sample prepared in this way would be by 10, 20 and 30 mg/100 ml higher in comparison with the basic concentration in the original sample. These additions served for the calculation of recovery, i.e. the recovery of applied methods for the MUC analyses.

CONCLUSION

The FT-MIR method for MUC determination in this study, by the application of control samples on the basis of native milk, eventually with artificial additions of urea, proved a good effectivity in terms of the reliability of results by the relevant calibration. In materials of IDF (International Dairy Federation) working group Lefier (1999) suggested to choose a specific enzymatic method as a reference method of MUC determination, especially for calibration indirect technical equipment. These results confirm the mentioned suggestion as relevant. Nevertheless, they also show that the use of a method with recovery proved locally in detail (addition or total one) is even more important than the strict choice of a method for avoidance of disproportions in the results (for common methods as well as for calibration). In the case of the convenient recovery value a less specific method (non-enzymatic) can also offer reliable calibration values.

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