Reliability of results of milk urea analysis by various methods using artificial milk control samples

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ABSTRACT: The milk urea concentration (MUC) is a respected indicator of the health and nutrition status of dairy cows. It is in relation to their reproduction performance, longevity and technological milk indicators. The accuracy of the interpretation of results depends on their reliability, which is so important. There are a lot of principles of MUC analyses. Their results can be affected by a number of interferential factors. Many disproportions were noticed for the above-mentioned reasons in laboratory practice. That is the reason why relevant result variation sources are studied. The goal of this paper was to search the relationships between different methods of MUC determination with the use of specifically modified samples on a milk basis with the absence of dissolved components such as lactose. The results of two methods (photometric BI with diacetylmonoxime and FT-MIR (mid infrared)) were disqualified for a large shift and variance of values, unsatisfactory recovery and paralysed relation to other methods (BI r = from 0.184 to 0.213; $P > 0.05$). Therefore the second BI method was retained in the evaluation, and it was probably a local defect in the performance at disqualification. Nevertheless, the procedure showed poorer recovery (75.5 ± 14.3%) and necessity for methodical modifications for support of result reliability such as increase in the number of calibration points as compared to the contemporary procedure. The results of FT-MIR method were strongly systematically displaced due to lactose absence in particular (by 33.824 ± 3.794 mg/100 ml). Nevertheless, the correlations with results of other relevant methods were tight (from 0.991 to 0.999; $P < 0.001$). The photometrical method with Ehrlich's agent (para-dimethylaminobenzaldehyde, EH) showed acceptable values of all the evaluated indicators of reliability. The specific Ureakvant method (UR; with conductivity difference measurement) showed the most proper results in combination with all the reliability indicators (recovery as much as 93.2 ± 10.2%; correlation from 0.989 to 1.0; $P < 0.001$; acceptable ratio of systematic and random error components). It is possible to use the tested specific standard samples for the control or calibration of all methods (BI, EH and UR) with the exception of FT-MIR.

Keywords: cow; milk; urea; method of analysis; result reliability; recovery; correlation

The urea as a product of liver detoxification of ammonium is in general an undesirable metabolite in body fluids of mammals, when its concentration exceeds the physiological limit. Its very low levels are nevertheless also undesirable as they indicate the nitrogen matter malnutrition. The control of

variability in milk urea concentration (MUC) is used for a diagnosis of the energy-nitrogen metabolism of cows (Kirchgessner et al., 1986; Gustafsson and Palmquist, 1993; Homolka and Vencl, 1993; Baker et al., 1995; Jonker et al., 1999; Hojman et al., 2004; Jílek et al., 2006; Strusiňska et al., 2006; Zhai et al., 2006). Both the high and the low levels of MUC may thus exert a negative effect on the health of dairy cows, their reproduction performance and longevity (Butler et al., 1996; Říha and Hanuš, 1999; Kubešová et al., 2005). Simultaneously, the high levels of MUC can also act by the deterioration of milk quality (Hanuš et al., 1993a,b). Recently, the genetical effects were also described. Miglior et al. (2006) established breeding values for urea nitrogen content in milk. They found relatively high coefficients of heritability h^2 = 0.38 for the 2nd and 0.41 for the 3rd lactation. Surprisingly the genetical correlations between lactations were also high (r = from 0.74 to 0.87 between the 1st and 3rd and between the 2nd and 3rd lactation). This might indicate a substantial genetical effect on differences in the ability of cows to utilize nitrogen from feed. For this reason the control of MUC is used in agricultural and nutrition laboratories in milk recording of dairy cows (individual milk samples) and in quality control of delivered raw milk (bulk milk samples). The results and their interpretation thus serve in consulting the nutrition of dairy cows and in prevention of their metabolism failure.

In spite of the above-mentioned facts, from methodical aspects there exists a number of analytical problems, when no reference method has been declared in a standard way yet (Lefier, 1999; Broutin, 2000, 2006a,b) while many different analytical principles are used (Lefier, 1999; Hanuš et al., 1997, 2001; Peterson et al., 2004; Broutin, 2000, 2006a,b) which under certain circumstances can be a source of significantly different results for many reasons. It is the reliability of the results that determines the relevant interpretation and practical application in the nutrition and health status control of dairy cows. For this reason this problem was a repeated target of some authors (Carlsson and Bergström, 1994; Hanuš et al., 1995; Herre, 1998; Klopčič et al., 1999; Peterson et al., 2004; Hering et al., 2007). Also in the Czech Republic it happened during the process of the merging of milk laboratories (2004 to 2006) that many bad interpretations occurred due to the cross application of many methods without a substantial accent on the unification and support of reliability of results by a consistent netting of relevant laboratories in the proficiency testing of analytical capability. The principles and advantages of netted laboratory proficiency testing (Leray, 1993; Wood et al., 1998; Hanuš et al., 1999, 2000) as well as evaluation of its results were discussed in many papers.

As mentioned above, the measurement of MUC as a physiology-health indicator can be done by several methods. FT-MIR technology is viewed as hopeful (Broutin 2006b; Roos et al., 2006) for the health state monitoring of milked ruminant females and for the coordination of prevention of their production and reproduction disorders linked with false nutrition and other bad technologies regarding their high milk yield. In spite of this, for milk urea determination direct methods are preferred, especially the specific ones (ureolytical and differential ones; Lefier, 1999; Broutin, 2000, 2006b), for example AFNOR (France), Eurochem (Italy) or Chemspec (Bentley Instruments, USA). Such is also the opinion of IDF in terms of the running selection of an official reference method (Lefier, 1999), where the specificity is the basic prerequisite. Here it is necessary to proceed by the difference methods, it means to compare the feedback of the reaction before and after ureolysis or during ureolysis and obtained parameters (mostly the difference) to calibrate according to known MUC. The most common difference measurements are provided photometrically (AFNOR, Chemspec), by the pH determination (Eurochem) or conductometrically (Ureakvant, Czech Republic). Still many other methods of MUC determination (specific or nonspecific ones) on the basis of different principles were described and evaluated (Rajamäki and Rauramaa, 1984; Oltner et al., 1985; Hanuš et al., 1997, 2001; Herre, 1998; Klopčič et al., 1999). A special problem is the preparation of milk urea standards for adequate calibration of different methods. This has been submitted to experiments and methodical examination, including the mutual result relationships of the methods, which are interpreted as potentially reference and other direct or indirect ones (for example filter MIR technology), frameworkly in the following fields:

– the preparation of calibration standards for different methods of MUC determination, potentially for urea determination in other body fluids (Wolfschoon-Pombo et al., 1981; Oltner et al., 1985; Hanuš et al., 1995, 1997);

– the examination, development, construction and validation of enzymatic sensors, reactors, and

potentially biosensors for the analysis of urea concentration in different biological materials, especially in milk (Guilbault and Montalvo, 1970; Sundaram, 1979; Thavarungkul et al., 1991; Sansubrino and Mascini, 1994; Koncki et al., 1995; Ciana and Caputo, 1996; Sheppard et al., 1996);
– the validation of the relationships of recently developed principles and methods of MUC determination or urea determination in blood to the current methods or to potentially reference ones, mostly specific ureolytical ones (Broutin, 2000);
– a notice of problems of recovery and reliability of urea determination under the conditions of mid filter transmission infrared technology (MIR) in comparison with validated methods, where the determination coefficient of accuracy was about 50% (Here, 1998).

The aim of this study was to evaluate the relationships between the currently used principles of methods of MUC determination in the Czech Republic, especially of new modern methods (measurement in the main area of infrared spectrum with use of Fourier transformations, FT-MIR; Lefier et al., 1996), on artificially constructed control samples on the milk basis and to deduce the relevant methodical recommendations for enhancement of reliability and improvement of practical interpretation of produced analytical results due to obtained theoretical relationships.

MATERIAL AND METHODS

Analytical methods

More detailed characteristic descriptions of the used methods can be found in previous papers (Hanuš et al., 1995, 1997, 2001; Klopčič et al., 1999; Hering et al., 2007). Here only a brief summary is given:
– the specific method Ureakvant (UR) is based on difference measurements of a change in the

Table 1. The results of analyses of urea concentration (mg/100 ml) in modified control samples by various methods and their reference values after the tests of outliers in two evaluations (I and II), total (before) and after the exclusion of FT-MIR and BI-2 methods

Method sample	FT-MIR	UR 1	UR 2	EH	BI 1	BI 2	UR 3	REF. I	REF. II	NA
1	67.4*	27.3	33.3	35.2	26.6	30.4	38.6	31.90	32.20	38.0
2	57.4*	21.0	26.0	26.3	16.8	30.7	30.3	25.18	24.08	29.0
3	76.7*	34.8	41.0	45.5	32.2	33.7	47.7*	39.14	40.24	47.0
4	38.0*	5.9	5.9	2.1	5.5	22.6*	7.4*	5.36	5.36	5.0
5	88.3	43.7	51.0	58.0	38.7	27.0	56.5	51.12	49.58	60.0
6	43.6*	11.6	13.3	10.3	10.8	25.4*	15.7*	12.34	12.34	13.0
7	70.7*	30.8	36.1	39.9	29.8	24.0	41.3*	33.65	35.58	42.0
8	49.3*	15.6	18.1	17.7	15.4	28.2*	21.0*	17.56	17.56	19.0
9	82.6*	40.6	47.7	53.9	39.1	33.9	53.1	44.72	46.88	55.0
10	55.0	18.8	22.7	22.6	18.6	44.7	26.1	29.79	21.76	24.0
x	62.9	25.0	29.5	31.2	23.4	30.1	33.8	29.08	28.56	33.2
sx	16.1	11.9	14.1	17.6	11.0	6.1	15.6	13.61	14.02	17.3
CV	25.5	47.6	47.8	56.5	47.3	20.2	46.1	46.80	49.1	52.1
Min.	38.0	5.9	5.9	2.1	5.5	22.6	7.4	5.36	5.36	5.0
Max.	88.3	43.7	51.0	58.0	39.1	44.7	56.5	51.12	49.58	60.0
$R_{\text{max.-min.}}$	50.3	37.8	45.1	55.9	33.6	22.1	49.1	45.76	44.22	55.0

FT-MIR = infrared method in the main area of spectrum with Fourier transformations; UR-1, UR-2 and UR-3 = specific, difference-conductometric method Ureakvant; EH = photometric method with Ehrlich's agent; BI-1 and BI-2 = photometric method BioLaTest; NA = urea weight; REF. I and REF. II = reference values after statistical exclusion of remote results (*Grubbs test on the level 95%) from total set (I) and after exclusion of remote results of methods (II)

electrical conductivity during the ureolytical hydrolysis of urea by urease and it was calibrated on a five-point scale of milk urea standards;
– the photometrical method BioLaTest (BI) is based on a change in colour by means of the reaction of diacetylmonoxime measured at 525 nm (Spekol 11, Carl Zeiss, Jena, Germany) and it was calibrated on one standard water sample;
– the photometrical method with Ehrlich's solution (EH) is based on a change in colour by means of the reaction of paradimethylaminobenzaldehyde measured at 420 nm (Spekol 11) and it was calibrated on the five-degree scale of standard water samples;
– the spectrophotometric method in the infrared area of spectrum (FT-MIR) on a Foss 6000 instrument (Foss Electric, Denmark) was calibrated on the ten-point scale of samples of native milk with different MUC according to the results of specific reference method (UR).

Design of experiment

The control samples (reference standards) were created on the basis of milk matrix with nearly zero urea concentration. The samples of native UHT milk were precipitated by acidification to pH 4.5 by hydrochloric acid and centrifuged. The supernatant with lactose, native urea and other soluble compounds was removed. Precipitated fat and protein fraction (especially casein) were mixed with pure water and repeatedly centrifuged. This process was repeated three times with the ratio of coagulation and washing water volumes 1:5. Coagulation was subsequently reconstituted in citrate phosphate buffer by pH 7.0. The obtained samples thus contained basic compounds of fat and protein milk phase by the absence of lactose

and other components of the soluble phase including urea. Known artificial weight additions were added into these modified samples to assess the recovery ability of examined analytical methods. In the experiment the series of 10 samples of modified milk was used (Table 1). The samples were immediately transported to selected laboratories under cold regime conditions. The MUC analyses of the samples (7 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 total recovery (yield) was then determined as a difference of the value measured after the addition from value 0 and 100% was formed by the value of the addition.

Evaluation of results

The results (Table 1) were evaluated by means of the calculation of recovery and of the determination of Euclidian distance for the reliability of laboratory results. The principles of these procedures are described in more details in previous papers of Leray (1993) and Hanuš et al. (2006). The mean deviations and variabilities of individual values of these deviations of the participants (laboratories, methods) from reference values and Euclidian distance (RE) of the laboratories from the origin (centre) were calculated. The correlation coefficients between combinations of participants were also calculated. In tables and figures it is possible to find positions of laboratories or methods in terms of the measure of measured result reliability. The discrimination limits of participation success in the proficiency testing in the tables and figures were derived in the following way:
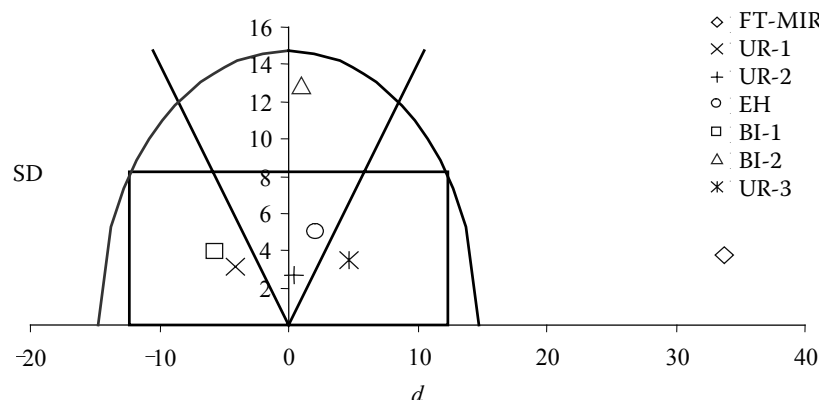

Figure 1. Euclidian distance of participants (I; *n* = 7) in MUC determination from origin (RE)

Table 2. General array of participants (I; *n* = 7) in the milk urea concentration (MUC) determination according to the Euclidian distance from origin (RE)

LAB.	*d*	SD	RE	*t*	*v*
UR-2	0.434	2.662	2.6971	0.52	NS
UR-1	−4.066	3.127	5.1294	4.11	**
EH	2.074	4.967	5.3826	1.32	NS
UR-3	4.694	3.502	5.8564	4.24	**
BI-1	−5.726	3.893	6.9241	4.65	**
BI-2	0.979	12.839	12.8763	0.24	NS
FT-MIR	33.824	3.794	34.0361	28.19	***

LAB. = laboratory (method, participant); *d* = mean deviation (difference, mg/100 ml); SD = variability of mean difference; RE = Euclidian distance; *t* = value t of the testing parameter of paired test; *v* = statistical significance (NS = *P* > 0.05; * = *P* ≤ 0.05; ** = *P* ≤ 0.01; *** = *P* ≤ 0.001)

– semicircle:
  (1) for the average difference (*d*) as 1.96-times multiplied of standard deviation value d of the set, it means 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 sd medians of the set and 1.65-times multiplied of standardized quartile estimate of standard deviation, it means at the conventional level 95%;
  (3) by the combination of both the previous (*d* and SD) limits on the basis of the mean the limit was created (semicircle in the graph) with discrimination ca 10%;
– tetragon:
  (4) for *d* as the value of one standard deviation in *d* set;
  (5) for SD as the sum of the means of the sd set and its standard deviation.

RESULTS AND DISCUSSION

Naturally, there also exist other ways of the preparation of standard samples than water solutions of urea with known additions, and thus concentrations (for recovery) or samples of native milk with determined MUC by another reference method. In native milk the MUC was depressed to 0 by addition of urease. The urease was consequently inactivated by the higher temperature. The artificial MUC were added into milk prepared in such a way (Hanuš et al., 1995). Nevertheless, such a procedure can be encumbered with possible residual activity of urease and the samples may not be stable under all circumstances. It is difficult to control of course. On the other hand, the advantage is the complete milk matrix, which decreases the risk of possible undesirable interferential effects on the reliability of the results in the calibrated methods (FT-MIR). However, for research in this
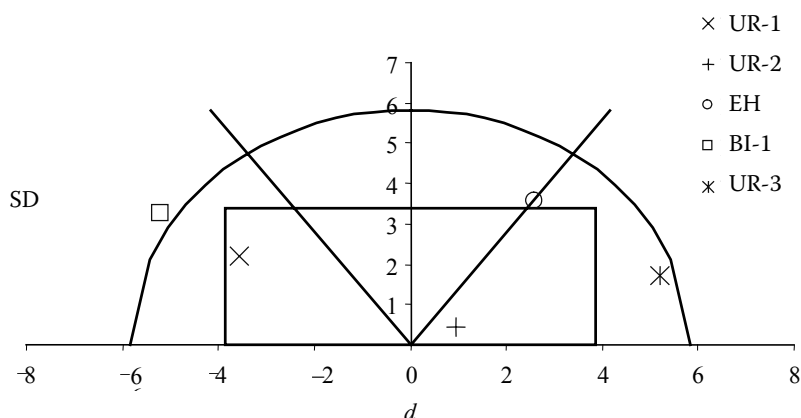

Figure 2. Euclidian distance of participants (II; *n* = 5) in MUC determination from origin (RE)

Table 3. The array of participants (II) in MUC determination after the exclusion of remote participants (*n* = 5) according to the Euclidian distance from origin (RE)

LAB.	*d*	SD	RE	*t*	*v*
UR-2	0.952	0.419	1.0401	7.18	***
UR-1	−3.548	2.180	4.1642	5.15	***
EH	2.592	3.588	4.4263	2.28	*
UR-3	5.212	1.705	5.4838	9.67	***
BI-1	−5.208	3.273	6.1511	5.03	***

work quite a different procedure was applied in the preparation of standard samples on the basis of milk matrix with the absence of soluble components. The mentioned results are related to this way of preparation. Regarding the various interferential effects Carlsson and Bergström (1994) did not find any effects on the MUC in individual udder quarters or any differences between the beginning and the end of milking (different fat contents), when the MUC was calculated in relation to the water phase of milk.

The basic evaluation (Table 1) by the RE method was carried out twice: once in the complete arrangement of the results (I) (Figure 1; Table 2) and then with just five participants (II; Figure 2; Table 3), when two of them were excluded. The exclusion was done on the basis of the significant positive difference of the results (*d*, systematic error) of FT-MIR method (33.824 ± 3.794 mg/100 ml; Table 2; Figure 1). Further due to the unsatisfactory correlation of the measured values in one of the methods BI-2 (from 0.184 to 0.213; *P* > 0.05; Table 4; Figure 3) in comparison with the values of the other methods, when the correlations between these methods were very tight (from 0.989 to 1.0; Table 4; *P* < 0.001) with the exception of the excluded method. Also the value of random error (SD;

Table 2; Figure 1) was the highest (±12.839 mg per 100 ml). Moreover, the value of total recovery in this participant (140.3 ± 122.1%) was unsatisfactory due to the false application of the method. It means both the average one and especially its variability (*CV* = 87.0%; Table 5). It is probably the same local discrepancy in the methodical procedure or its qualitative assurance as in the previous paper of ours (Hering et al., 2007), because the second BI-1 method was not excluded from the set (II) in this case (Table 3; Figure 2).

Due to the exclusion of the mentioned substantially significant interferential effects (Figure 2; Table 3) the second evaluation (II) is thus *espressivo* more effective than the first one regarding the fact how to evaluate the reliability of the results of the analytical methods by the given RE procedure. It is logically possible to observe lower discrimination limits and higher level of evaluation in demands on analytical procedures. Owing to the fact that the second method BI-1 had good correlations (from 0.989 to 0.996; *P* < 0.001; Table 4), although with relatively higher negative d (systematic error; −5.208 mg/100 ml; Table 3), it is possible to state that the method itself is applicable by the adequate calibration. Also the total recovery value of this method (75.5 ± 14.3%; *CV* = 18.9%) was lower due to the probable negative
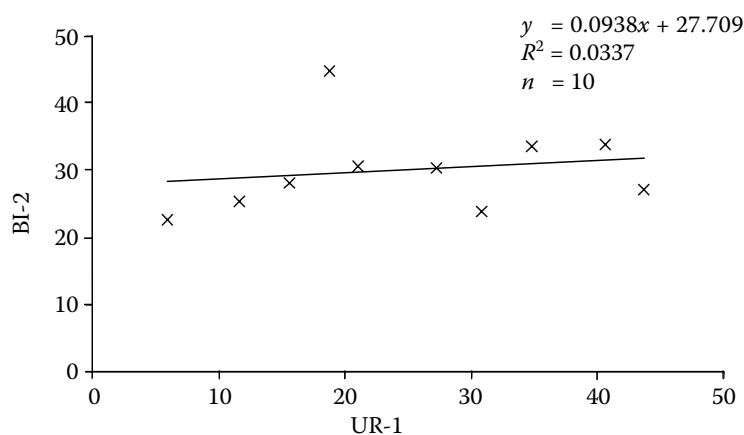

$y = 0.0938x + 27.709$
$R^2 = 0.0337$
$n = 10$

Figure 3. The most suited linear regression relationship (*r* = 0.184; *P* > 0.05) between the methods of MUC determination (mg/100 ml), UR-1 × BI-2

Table 4. Correlation coefficients of linear regression of the relationships between the methods of MUC determination

	FT-MIR	UR-1	UR-2	EH	BI-1	BI-2
UR-1	0.999					
UR-2	0.998	0.999				
EH	0.998	0.999	1.000			
BI-1	0.991	0.993	0.991	0.992		
BI-2	0.188	0.184	0.207	0.202	0.209	
UR-3	0.997	0.997	0.999	0.999	0.989	0.213

$P \leq 0.001$ except BI-2 to the others, $P > 0.05$

calibration shift (Table 5). The second case of BI-1 method suggests that the method can work correctly. The improvement can be achieved by using a higher number of calibration samples instead of just one. Probably it would eliminate the marked random effect when the potential error of one standard becomes the systematic error of linked analyses and its higher probability of occurrence the source of the random error of the method.

The modern FT-MIR method which is the most important subject of the experimental comparison revealed strongly displaced results. It means the large systematic error (d) in the first evaluation. This difference however expressed a very constant character, when the coefficient of variation was ±11.2%. It was probably a consequence of the absence of soluble milk components, especially of lactose, and also dissolved minerals and citrates, which originated due to manipulation with samples during the preparation of artificial milk standards. Consequently the total method recovery increased disproportionally including its variability 258.8 ± 185.5% (Table 5; $CV = 71.7\%$) while the correlations with the results of other relevant methods were tight (Table 4; from 0.991 to 0.999; $P < 0.001$). For

this reason and simultaneously in spite of these results this method was excluded from the second evaluation (Figure 2 and Table 3). If the otherwise relatively constant factor (29.7 ± 1.6 mg/100 m, differences between weights and measurement results, $n = 10$, $CV = 5.4\%$) was subtracted from measurement results, also the total recovery would be satisfactory although its variability would be slightly higher (106.6 ± 21.2% and $CV = 19.9\%$) and also the reliability of the MUC results would be better (in expression of d in Figure 2; 4.64 ± 2.32 mg/100 ml as expressed to the reference values II, Table 1). Such complicated treatment of the results is nevertheless probably behind the limits of being analytically admissible. Peterson et al. (2004) determined the recovery for Foss 6000 system (FT-MIR) 95.4 ± 10.1%, for Bentley 92.1 ± 2.8%. For this reason the mentioned recovery could not be confirmed in this paper. Peterson et al. (2004) also confronted some of the applied methods for determination of milk urea nitrogen concentration (MUN): when the initial concentration of urea nitrogen increased, the recovery decreased using the Bentley system and CL-10 system. Increased milk fat resulted in the recovery depression using the Foss 6000 system. In
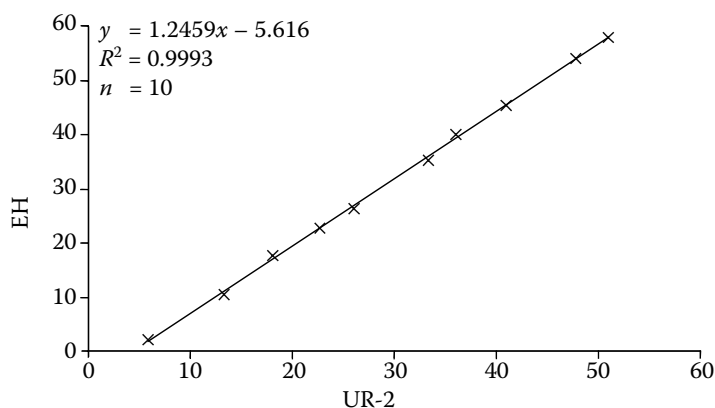

$y = 1.2459x - 5.616$
$R^2 = 0.9993$
$n = 10$

Figure 4. The narrowest linear regression ($r = 1.0$; $P < 0.001$) between the methods of MUC determination (mg/100 ml), UR-2 × EH

Table 5. Average values of total recovery (%) and their variability for MUC by ten modified samples of milk

Method	R	CV
FT-MIR	258.8 ± 185.5	71.7
UR-1	80.6 ± 14.3	17.7
UR-2	93.2 ± 10.2	10.9
FH	87.8 ± 17.0	19.4
BI-1	75.5 ± 14.3	18.9
BI-2	140.3 ± 122.1	87.0
UR-3	108.5 ± 15.9	14.7
NA	100	

R = recovery in % (x ± sx); CV = coefficient of variability in (%); 100% = weight of urea (NA, Table 1)

4 out of 5 methods the MUN recovery was not connected with specific milk components. The MUN recovery was not consistent for laboratories using the methods Foss 4000 and 6000 and the application of these systems can result in MUN overestimation or underestimation.

The EH method showed out relatively very hopeful values of total recovery (87.8 ± 17.0%, CV = 19.4%; Table 5) although it had a slightly higher positive systematic error in both evaluations 2.074 and 2.592 mg/100 ml also with the higher value of random error SD (Figures 1 and 2; Tables 2 and 3). However, the correlations of the results with the results of other relevant methods were tight (from 0.992 to 1.0; $P < 0.001$; Table 4).

None of the tested methods reached positive parameters of the specific UR method, which in two out of three cases (one worse result was probably a local case) showed in fact the best combinations of the results in the total recovery (Table 5; UR-1 80.6 ± 14.3%, CV = 17.7; UR-2 93.2 ± 10.2%, CV = 10.9%; UR-3 108.5 ± 15.9%, CV = 14.7%), correlations (Table 4; from 0.989 to 1.0; $P < 0.001$; Figure 4), systematic and random error (Figures 1 and 2; Tables 2 and 3) and this to artificial additions, to one another and also to other relevant methods. Some variability of total recovery is probably a consequence of current calibrations of the instruments and of functional states of individual reactors of particular equipments. Generally, advantageous results of the UR method are in fact in accordance with former positive findings (Klopčič et al., 1999; Hanuš et al., 2001; Hering et al., 2007). Comparable positive results of the result reliability of MUC analyses were also described by Broutin (2000,

2006a,b) for the automatic specific (application of urease) method Chemspec (Bentley Instruments, USA) with photometrical output of measurements of the colour intensity in modified Berthelot's reaction (Patton and Crouch, 1977), which is proportional to the ammonium concentration.

In the case of specific control samples the presented results suggest simultaneously good effectivity in terms of the result reliability of the methods UR, EH and FT-MIR. This means also a good state regarding the possibility of providing information for correct practical interpretations in the field of nutrition and health state control of dairy cows. On the basis of results of this paper and the previous one (Hering et al., 2007) it is possible to state that the BI method requires higher methodical support of result reliability assurance than it has been until now. The results suggest also that the carrying out of such step is not excluded.

CONCLUSION

In this paper the method FT-MIR for MUC determination showed relatively paralysed effectivity in terms of result reliability (also by relevant calibration) under the circumstances of work with artificially modified control samples on the milk basis. Such a level of unreliability was nevertheless determined just by the relatively constant systematic shift of MUC values (enhancement), which was the result of the absence of some soluble milk components, especially lactose. But such samples cannot be used for the control of this method in comparison with the other methods. In the IDF evaluation (International Dairy Federation) Lefier (1999) suggested to use a specific enzymatic method especially for the calibration of the indirect instrumental equipment. These results confirm this suggestion, but they further show that it is more important for the prevention of result discrepancies to use a method with locally assured recovery (addition or total) than a strict choice of the method principle. In the case of good recovery obtained also by a less specific method (without urease) this can offer relevant reference values for the calibration of indirect technique. If modified standard samples on the milk basis are used, it is not commonly possible to use them as control or calibration ones for FT-MIR method. For such a possibility it would be needed to estimate very precisely the eventual interferential effect of modification on MUC determined by the FT-MIR and its variability

and to accept these values for appropriate control or calibration calculations. Quality managers in accredited milk laboratories should pay an appropriate attention to the theoretical and technical performance of methods for MUC determination and their validations minimally in intentions of the results and recommended procedures found here and in a previous paper (Hering et al., 2007). It is necessary to do it in this way in order to reach a necessary positive impact on field interpretations of reliable results in dairy advisory service.

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