

Analytic Validation of an Infrared Milk Urea Assay and Effects of Sample Acquisition Factors on Milk Urea Results

S. M. Godden,* K. D. Lissemore,† D. F. Kelton,†
J. H. Lumsden,‡ K. E. Leslie,† and J. S. Walton§

*Department of Clinical and Population Sciences,
University of Minnesota, St. Paul, MN 55108

†Department of Population Medicine, ‡Department of Pathobiology,
and §Department of Animal and Poultry Science,
University of Guelph, Guelph, ON, Canada N1G 2W1

ABSTRACT

The objective of this study was to determine if milk samples, as they are routinely collected by Ontario Dairy Herd Improvement, would yield accurate milk urea results with an infrared assay. This investigation involved analytic validation of the infrared assay and assessment of the effect of DHI routine sample acquisition factors on milk urea results.

Analytic validation of an automated milk urea assay was performed by assessing the relative accuracy and precision of milk urea results produced by the Fossomatic 4000 Milk Analyzer, an infrared method of analysis, compared with the Eurochem test, an accepted reference method. Results indicated that, when interpreted at the group level, milk urea results between the infrared method and the reference test were in good agreement. The two tests shared a similar and high level of precision.

Milk urea concentrations obtained from composite (metered) milk samples, and not quarter stripping samples, were most representative of concurrent serum urea concentrations. The addition of bronopol preservative did not result in a numerically important change in milk urea concentrations. Storage of preserved metered milk samples for up to 4 d at either room temperature or by refrigeration, or for up to 3 d by freezing, did not result in changes in milk urea concentrations.

We concluded that milk samples, as they are routinely collected and handled by DHI, are suitable for measurement of milk urea concentrations with the infrared method of analysis if data are interpreted at the group level.

(Key words: infrared milk urea assay, dairy herd improvement, sample acquisition)

Abbreviation key: DHI = Dairy Herd Improvement, IR = Infrared, MU = milk urea (mmol/L), P_c = concor-

dance correlation coefficient, SU = serum urea (mmol/L), UHT = ultra-high temperature. **Conversion formula:** MUN (milk urea nitrogen, mg/dl) = MU (milk urea, mmol/L) \times 2.8.

INTRODUCTION

Although the technology to measure serum urea (SU) concentrations has been available for many decades (19), this approach has not been widely adopted, in part because of the time, inconvenience, and expense of collecting and analyzing large numbers of blood samples. The same is true of chemical (or lab bench) methods available for measuring urea concentrations in milk samples (MU). The early 1990s witnessed the introduction of automated instrumentation that uses infrared spectrophotometric methods to estimate the concentration of urea in milk samples. Because individual cow milk samples are routinely collected by DHI, the use of infrared (IR) technology offers a rapid and inexpensive means of measuring milk urea concentrations.

Before any new test is adopted by industry, its performance should be rigorously and impartially evaluated under laboratory and field conditions. The first step of this process examines how well the new test performs under laboratory conditions compared with other existing methods. Two important characteristics to evaluate are analytic accuracy and analytic precision. Analytic accuracy is the agreement between the best estimate of a quantity, as determined by a new test, and its 'true' value, as determined by a definitive reference method (6). Analytic precision is the agreement between replicates (i.e., the ability of the analytical method to produce the same value for replicate measurements of the same sample) (6). It is important that these test characteristics are known and considered when the practical applications of the test under field conditions are later assessed.

Also important is whether the intended specimen acquisition process will affect the quality of the test results. Factors that may affect results must be understood, not only for evaluating the suitability of a tech-

Received June 14, 1999.

Accepted September 26, 1999.

Corresponding author: S. Godden; e-mail: godde002@tc.umn.edu.

nology for its intended use, but also for later designing and implementing a quality assurance program (6). Previous research (8, 10, 17) has reported on the effects of some sample acquisition factors, including the effect of sample type (quarter stripping vs. composite), addition of preservative, storage temperature, and duration of storage on MU results. However, these studies were performed by indirect chemical methods of analysis, not the infrared method that was adopted by many DHI laboratories.

Ontario DHI installed the Fossomatic 4000 Milk Analyzer (Foss Electric, Brampton, ON) in the spring of 1995, but did some analytic and field validation before commercial MU testing was made available to Ontario dairy producers. The objective of this study was to determine if milk samples that are routinely collected by Ontario DHI would yield accurate MU results with an IR assay. The first step was to evaluate, with field samples, the relative accuracy and precision of an infrared MU assay compared with an accepted reference method. The second step was to evaluate the effect of various factors related to sample collection and storage on MU results with the IR method of analysis. Factors examined included the effect of sample type [quarter stripping vs. composite (metered) sample]; the effect of bronopol preservative; and the stability of MU concentrations over time when samples were stored at room temperature, refrigerated, or frozen.

MATERIALS AND METHODS

Milk samples collected by Ontario DHI field staff were handled consistently. Composite (metered) milk samples, representative of the entire milking of individual cows, were transferred into sample vials, each of which contained a tablet of bronopol preservative to inhibit the growth of bacteria and yeast. When the herd test was complete, milk samples were transported, via courier, to the Ontario DHI laboratory. Milk samples were not refrigerated during transport. Samples may occasionally freeze if left in courier trucks overnight during the winter months. Upon delivery to the laboratory, samples were refrigerated until they were analyzed for MU and other milk components. Samples were usually analyzed within 1 to 2 d of collection, although over a long weekend, samples might not have been analyzed for up to 4 d after collection.

Analytic Validation of an Infrared Milk Urea Assay

Analytic accuracy. Eighty-nine metered milk samples from individual cows were selected from among routine herd test samples submitted to the Ontario DHI laboratory. Each milk sample was divided into paired

duplicate samples. One of each pair of duplicate samples was then analyzed for MU (mmol/L) with either the Fossomatic 4000 Milk Analyzer (Foss North America, Brampton, Ontario) or the Eurochem test (Foss North America, Brampton, Ontario), a differential pH, and recognized reference, method.

Overall agreement in MU results between the two test methods was assessed by two different statistical approaches. The first approach was to calculate the concordance correlation coefficient (P_c) to estimate the overall agreement with continuous measures (22). The second approach used was the Bland and Altman method. This method involved plotting the mean of the paired measurements (x-axis) against their difference (y-axis). The 95% confidence interval around the mean of the differences was calculated and superimposed on the plot. The number of points that fell within the 95% confidence intervals (or limits of agreement) were then observed and recorded (4).

Analytic precision. Two separate studies were performed to estimate the CV, a measure of test precision, for the Fossomatic 4000 MU assay. In the first study, MU concentrations were measured in replicate milk samples derived from the same original batch of ultra-high temperature (UHT) treated milk. One UHT milk sample was included in all regular runs of the MU assay for a period of 14 d. Over the 14 d, averaging 4 runs per day, 55 MU were determined from the same batch of UHT milk. The MU results were used to calculate the CV (24).

In the second study, composite milk samples were collected from milk meters at the time of the regular morning milking, from 24 Holstein cows of mixed parity and stage of lactation at a University of Guelph Dairy Research Station. Each milk sample was split to create four identical replicates. All replicate samples were preserved with a bronopol tablet (2-bromo-2-nitro-propane-1,3 diol: 6 mg/tablet: D & F Control, San Ramone, CA), transported directly to the Ontario DHI laboratory, refrigerated overnight, and then analyzed for MU concentrations the following day. Two of each pair of four replicate samples were analyzed with the Fossomatic 4000 milk analyzer; the other two samples were analyzed by the Eurochem test. The MU results were then used to calculate a separate CV for each test method (23).

Factors Related to Sample Collection and Handling

Sample type. Milk and blood samples were collected from each of 26 Holstein cows of mixed parity and stages of lactation at the regular morning milking. Animals were not offered the morning feeding until after the morning milking. Premilking quarter stripping (fore-

stripping), composite (metered), and postmilking quarter stripping (poststripping) milk samples were collected from each cow into separate vials, each containing a tablet of bronopol preservative. A blood sample was collected from the coccygeal vein into a Vacutainer tube within 5 min of the completion of milking for each cow. All milk samples were submitted directly to the Ontario DHI laboratory for same-day analysis of milk constituents, including total protein percent, butterfat percentage, SCC, and MU concentration using the Fossomatic 4000 Milk Analyzer. Serum from the blood samples was submitted to the Clinical Pathology Laboratory at the Ontario Veterinary College (University of Guelph, Guelph, ON) for measurement of SU concentration with the Dacos Biochemistry Analyzer (Coulter Electronics, Hialeah, Florida).

Analysis of variance and contrast analysis (20) were used to determine if, and how, the sample content of milk urea, butterfat, total protein, and somatic cells varied by the type of sample collected. Bonferonni's correction was used to calculate the critical P value for the contrast analysis to account for the three separate contrasts being performed for each milk component [$P_{\text{bonferonni}} = (P_{\text{original}}/3) = (0.05/3) = 0.017$] (22). Milk urea results from each of the three types of milk samples [forestripping, composite (or metered), and poststripping] were compared with the concurrent SU measurement, using the concordance correlation coefficient as a measure of overall agreement, to determine which milk sample type yielded MU concentrations that were most representative of the concurrent SU concentration (22).

Bronopol preservative. Composite (metered) milk samples were collected from each of 26 Holstein cows of mixed parity and stage of lactation at the regular morning milking. Each sample was split into two replicate samples, one of which was left unpreserved. A tablet of bronopol preservative was added to the other. All milk samples were submitted directly to the Ontario DHI laboratory for analysis of MU concentrations with the Fossomatic 4000 Milk Analyzer. Milk urea measurements from preserved samples were then compared with those of the nonpreserved samples using a paired *t*-test.

Storing samples at refrigerated, frozen, or room temperatures. Composite (metered) milk samples were collected from each of 23 Holstein cows at the regular morning milking. Each sample was divided into eight identical replicates, each of which was preserved with a bronopol tablet. Four of these samples were stored by refrigeration (4°C) while the other four samples were stored by freezing (−18°C). One of each sample type (refrigerated or frozen) was submitted for MU analysis with the Fossomatic 4000 Milk Analyzer after

storage for 2, 3, 4, or 7 d. Analysis of variance (20) was used to determine the effect of time in storage on MU concentrations, for both refrigerated and frozen samples.

In a second study, composite (metered) milk samples were collected from each of 24 Holstein cows at the regular morning milking. Each sample was divided into eight identical replicates, each of which was preserved with a bronopol tablet. Four of these samples were stored by refrigeration (4°C), and the other four samples were stored at room temperature (21°C). One of each sample type (refrigerated or room temperature) was submitted for MU analysis with the Fossomatic 4000 Milk Analyzer, after storage for either 1, 2, 3, or 4 d. Analysis of variance (20) was used to determine if MU concentrations varied over time by method of storage (refrigeration or room temperature).

RESULTS

Analytic Validation of an Infrared Milk Urea Assay

Analytic accuracy. The means and distributions of MU concentrations for the 89 samples analyzed were similar between the Fossomatic 4000 Milk Analyzer (mean = 4.11 mmol/L, standard error = 0.10, minimum = 1.61, maximum = 6.79) and the Eurochem test (mean = 4.24 mmol/L, standard error = 0.12, minimum = 2.00, maximum = 7.92). The mean difference between the two tests was 0.13 mmol/L of MU (standard deviation = 0.55). The 95% confidence limits (lower and upper limits of agreement) for the point estimate of the mean difference was calculated to be (−0.96, 1.24) mmol/L of MU. The concordance correlation coefficient was 0.86 (Figure 1). By using the Bland and Altman method of assessing agreement, approximately 95% of data points were observed to fall within the upper and lower limits of agreement.

Analytic precision. In the first study of 55 replicate samples of UHT milk analyzed over a 14-d period, the mean and standard deviation MU concentrations were 6.52 and 0.23 mmol/L, respectively. The CV was 3.44%. In the second study of 24 sets of replicate milk samples analyzed on a single day, the mean, standard deviation, minimum, and maximum MU concentrations measured by the Fossomatic 4000 milk analyzer were 5.52, 0.78, 4.57, and 7.96 mmol/L, respectively. The CV for the Fossomatic 4000 milk analyzer and the Eurochem test were 4.85 and 2.65%, respectively.

Factors Related to Sample Collection and Handling

Sample type. Analysis of variance indicated that milk urea concentration (mmol/L), butterfat percent, total protein percent, and somatic cells (×1000/ml) all

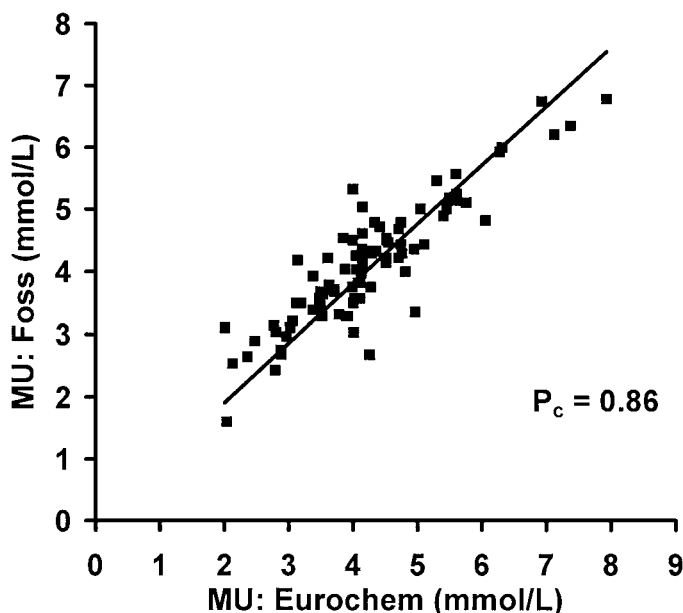


Figure 1. Milk urea (MU) results from the Fossomatic 4000 Milk Analyzer vs. the Eurochem test. P_c = concordance correlation coefficient.

differed by type of milk sample ($P < 0.05$ for each component). Contrast analysis indicated that urea and butterfat content were both lowest in forestripping samples. Butterfat content and somatic cells were highest in poststripping samples. Total protein was lowest in poststripping samples (Table 1). Urea concentrations were positively associated with butterfat concentration

Table 1. Variation of milk components among forestripping, composite, and poststripping milk samples

Milk Component	Sample type	Mean	SD	Min	Max
Butterfat, % (n = 26)	Forestripping	1.4 ^a	0.6	0.7	2.5
	Composite	3.4 ^b	0.7	2.5	5.0
	Poststripping	8.8 ^c	2.6	4.3	13.8
Protein, % (n = 26)	Forestripping	3.2 ^a	0.2	2.8	3.8
	Composite	3.2 ^a	0.2	2.7	3.8
	Poststripping	2.8 ^b	0.3	2.2	3.4
Somatic cells, × 1000/ml (n = 26)	Forestripping	85 ^a	83	18	331
	Composite	67 ^a	57	19	278
	Poststripping	244 ^b	282	20	940
Urea, mmol/L (n = 26)	Forestripping	3.37 ^a	0.93	1.71	4.89
	Composite	4.14 ^b	0.69	2.93	5.39
	Poststripping	3.66 ^{a,b}	2.15	0.29	9.50

^{a,b,c}Means within component with different superscripts differ ($P_{\text{bonferonni}} < 0.017$).

(coefficient = 0.41, $P < 0.01$) and negatively associated with SCC (coefficient = -0.011 , $P < 0.0001$).

Results from a comparison between serum urea and milk urea concentrations in each of the three types of milk samples (composite, forestripping and poststripping) are presented in Table 2.

Bronopol preservative. Milk urea concentrations were lower in unpreserved milk samples than in preserved samples (mean difference = -0.089 mmol/L, standard error = 0.036, $P < 0.05$).

Storing samples at refrigerated, frozen, or room temperature. Milk urea concentrations remained unchanged in preserved samples that were refrigerated for up to and including 4 d prior to analysis ($P > 0.05$), but were elevated in samples that were refrigerated for 7 d ($P = 0.0001$) (Table 3).

Milk urea concentrations remained unchanged in preserved samples that were frozen for up to 3 d after collection ($P > 0.05$) but were elevated in samples which were frozen for 4 or 7 d ($P < 0.05$) (Table 3). Milk urea concentrations were not different between matched pairs of refrigerated and frozen samples that were analyzed on d 2, 3, 4, or 7 postcollection ($P > 0.05$).

In the second study it was found that there was no interaction present between the effects of method of storage (room temperature vs. refrigeration) and duration of time in storage (1 to 4 d) ($P > 0.05$). As such, data for all 4 d were combined, for each individual animal, to calculate one mean MU concentration for each treatment (refrigeration vs. room temperature). The means, standard deviations, and ranges of MU concentrations for the 23 samples stored by refrigeration and at room temperature were: 5.40 ± 0.77 , 4.04 to 7.75 mmol/L, and 5.43 ± 0.79 , 3.93 to 7.93 mmol/L, respectively. Milk urea concentrations were not different in samples stored by refrigeration as compared with samples stored at room temperature for a storage period of up to and including 4 d ($P > 0.05$).

DISCUSSION

Analytic Validation of an Infrared Milk Urea Assay

Analytic accuracy. Analytic accuracy is defined as the agreement between the best estimate of a quantity, as determined by the new test, and its “true” value, as determined by a definitive method or an accepted reference method (6). Because there is no gold standard test for MU measurement, the Eurochem test was selected as an accepted reference method. The US DHIA has named the Eurochem test as the accepted reference method for MU analysis in North America, and it has been named as one of two suitable reference methods

Table 2. Comparison of milk urea (MU) concentrations from forestripping, composite, and poststripping milk samples with concurrent serum urea (SU) concentrations

	Sample type used for milk urea determination		
	Forestripping (n = 26)	Composite (n = 26)	Poststripping (n = 26)
Mean difference (SU – MU) (mmol/L)	0.83	0.057	0.54
Std. error	0.21	0.18	0.47
P value	0.001*	0.76	0.30
Pearson's correlation coefficient (r)	0.51	0.64	0.06
P value	0.008**	0.004**	0.78
Concordance correlation coefficient (P _c)	0.38	0.55	0.05

*Mean difference significant at $P < 0.05$.

**Pearson's correlation coefficient significant at $P < 0.05$.

by the International Dairy Federation (14) (P. Sauve, 1998, Canadian Laboratory Accreditation Program and the National DHIA Quality Certification Program, personal communication).

Our results indicated that there was good overall agreement between the two tests at the group level. However, for individual cow samples, we observed a large 95% confidence interval around the mean difference of 0.14 mmol/L (95% C.I. = (-0.96, 1.24)). We interpreted this as a relative lack of agreement between the two tests for individual cows.

One explanation for the analytic variation in results from individual cow milk samples is the way in which the IR method produces a urea estimate. The IR method measures the amount of light absorbed at a wavelength that detects urea nitrogen to quantify the concentration of urea in the sample. However, other milk components will also absorb some light at the urea wavelength. Up to nine different milk components, including butterfat, lactose, true protein, citrate, and somatic cells, can interfere with the urea measurement. Some of these interfering components are known to have a positive effect on the urea estimate (e.g., butterfat content), while others have a negative effect (e.g., SCC). As such, the

IR method must also measure the amounts of these interfering components at other wavelengths, then make a mathematical adjustment to control for their concentrations in the sample to produce an indirect estimate of the concentration of urea nitrogen. Approximately 45 to 50% of the urea estimate comes from the actual optical reading of urea in the sample, while the other 50 to 55% comes from the mathematical adjustment for concentrations of other interfering components (H. Hansen, 1997, Foss Electric, Hillerød, Denmark, personal communication). Because the concentrations of each of these interfering components tend to vary among cows, the IR method tends to produce a different urea estimate, even when samples from different cows may actually have the same true urea value.

This analytic variability will inevitably contribute to the large difference observed among MU results from individual cows (16, 21). Researchers have recommended that the problem of variation in MU results among individual cows may be removed if MU data produced by IR testing is interpreted at the group level (5, 7, 11, 12, 16, 21). The findings of this study lend further support to these recommendations.

Analytic precision. A test performance characteristic such as precision should, ideally, be assessed over a range of concentrations and over time. Because the CV of a test is only a relative measure of precision, it is most meaningful compared with that of other accepted or currently used test methods. The first study, which accounted for both within-day and between-day variability in test performance, indicated a high level of precision for the Fossomatic 4000 Milk Analyzer (CV = 3.44%). Unfortunately, we determined this for one MU concentration, and, due to the lack of availability of an accepted reference method at the time of this particular study, we did not compare it with the relative precision of a reference method. The second study,

Table 3. Effect of time in storage on milk urea concentrations (mmol/L) when storing samples by refrigeration or by freezing.

Storage period	n	Refrigeration		Freezing	
		Mean	SD	Mean	SD
2 days	23	12.18 ^a	1.72	12.37 ^c	1.53
3 days	23	12.29 ^a	1.88	12.41 ^c	1.28
4 days	23	12.49 ^a	1.67	13.35 ^d	1.45
7 days	23	12.90 ^b	1.62	13.50 ^d	1.61

^{a,b}Means within column with different superscripts differ from value on d 2 ($P < 0.05$).

^{c,d}Means within column with different superscripts differ from value on d 2 ($P < 0.05$).

which considered a range of MU concentrations, showed that the Fossomatic 4000 Milk Analyzer shared a similar and high level of precision with that of the Eurochem test ($CV_{\text{Foss}} = 4.85\%$, $CV_{\text{Eurochem}} = 2.65\%$).

The analytic validation studies described above were performed between 1995 and 1996 and yielded information that described the relative accuracy and precision of the Fossomatic 4000 MU assay, which was currently in use at the Ontario DHI laboratory. Because this study did not compare results among different DHI laboratories, the accuracy and precision information produced from this study should not be extrapolated to MU data produced by other DHI laboratories. All North American DHI laboratories participate in an ongoing laboratory quality certification program designed to monitor and ensure the quality of milk component data. Since the fall of 1998, this program has begun to monitor and ensure the quality of the MU data produced by all Canadian and US DHI laboratories that offer MU testing (P. Sauve, 1998, Canadian Laboratory Accreditation Program and the National DHIA Certification Program, personal communication).

Factors Related to Sample Collection and Handling

Sample type. Although composite (metered) milk samples are routinely collected by Ontario DHI for milk recording purposes, the influence of sample type (forestripping vs. composite vs. poststripping) was investigated because of inquiries made by some producers whether quarter-stripping samples could be submitted.

In evaluating the effect of sample type on MU concentrations, we compared MU concentrations to concurrent SU concentrations. The validity of this comparison is based on studies that have previously demonstrated a strong positive correlation between MU and SU concentrations. Reported Pearson's correlation coefficients range from 0.73 to 0.98 (2, 5, 9, 16, 18). Also, producers are using MU data as a surrogate measure of the systemic urea concentration (i.e., SU). Thus, assessing the agreement between MU and SU concentrations, as performed in this study, was important. Ideally, MU concentrations determined by the Fossomatic 4000 Milk Analyzer for each of the three types of milk samples would also have been compared with MU concentrations as measured by a recognized reference method. Such a method was not available at the time that this particular piece of research was performed.

The highest concordance correlation coefficient, a measure of overall agreement, was found between SU and MU concentrations from composite (metered) milk samples ($P_C = 0.55$). Poorer overall agreement was observed between serum urea concentrations and milk urea concentrations from either forestripping ($P_C =$

0.38) or poststripping milk samples ($P_C = 0.05$). The reasons for poorer agreement between SU and MU concentrations for the latter two sample types is attributed to some combination of those factors that contribute to the concordance correlation coefficient calculation, including greater mean differences in urea concentrations, greater variation around the mean differences, and weaker or nonexistent associations as measured by Pearson's correlation coefficient (refer to Table 2).

The results of this study suggest that composite (metered) milk samples, and not quarter stripping samples, should be submitted if samples are to be analyzed for MU by routinely calibrated IR analysis methods. These results differ from results of previous studies that, by indirect chemical methods of analysis, reported that both stripping or composite milk samples were suitable for MU analysis (10). Poststripping samples have much higher and more varied butterfat contents than do composite (metered) milk samples (Table 1). Because the equipment was originally calibrated with composite milk samples with more normal butterfat concentrations, the computer algorithm was unable to adequately control for the interfering effect of extremely high and varied butterfat contents when producing a urea estimate for poststripping milk samples. Thus, the MU estimates produced from poststripping samples were highly variable and did not agree at all well with concurrent SU concentrations, as was indicated by a very poor concordance correlation coefficient ($P_C = 0.05$). Gustafsson and Palmquist (10) may have produced different results because milk components which vary with sample type are unlikely to have an interfering effect on producing urea measurements when using indirect chemical methods of analysis. Although the interfering effect of butterfat was one possible source of variation discussed here, it is possible that SCC and several other interfering milk components contributed to the variability in MU concentration seen with different sample types.

Bronopol preservative. The Fossomatic 4000 Milk Analyzer at Ontario DHI was initially calibrated with bronopol-preserved composite milk samples, as it was known that DHI milk samples will routinely contain a bronopol tablet. The effect of bronopol preservative was evaluated to quantify the difference in urea estimates between preserved and nonpreserved samples. This evaluation was important to determine the potential effect on MU results if submitting an atypical nonpreserved milk sample for IR analysis.

In previous studies, the addition of bronopol or sodium azide preservative to milk samples did not significantly alter MU concentrations when samples were analyzed by indirect chemical methods (8, 15, 17). In contrast, this study, using IR analysis, found that milk

samples without bronopol preservative had significantly lower MU concentrations than did preserved samples ($P < 0.05$). However, given the numerically small mean difference observed (mean difference = -0.089 mmol/L, standard error = 0.036 mmol/L), the addition or lack of bronopol preservative to milk samples is unlikely to be an important consideration when interpreting MU results produced by IR analysis through routine DHI testing.

Storing samples at refrigerated, frozen, or room temperatures. The purpose of this series of studies was to investigate the stability of MU concentrations in preserved metered milk samples that were subjected to storage periods and conditions that were typical of what might be encountered by samples as they are routinely collected and handled by DHI field and laboratory staff. Results of the first set of studies indicated that MU concentrations were unchanged in preserved samples that were refrigerated for up to and including 4 d prior to analysis. Previous studies with indirect chemical methods of analysis reported that MU concentrations remained unchanged for at least 7 d in a refrigerator (4°C) (8, 15, 17). The results of this study indicate that preserved composite milk samples, which are transported promptly after collection, stored by refrigeration, and then analyzed within 4 d of collection are suitable for MU analysis.

Milk urea concentrations were unchanged in samples frozen for up to 3 d, but were elevated in samples frozen for 4 or 7 d (-18°C). Previous studies using indirect chemical methods of analysis found that MU concentrations remained unchanged after at least 7 d in the freezer (-18 to -20°C) (8, 15, 17). Different findings between studies may be at least partly attributed to changes in the content of other milk components that are known to be affected by freezing. Previous studies have demonstrated that freezing had the effect of lowering butterfat percentage (13, 25). Studies have reported inconsistent findings as to the effect of freezing on SCC. Timms et al. (25) reported that freezing increased SCC, while Barkema et al. (3) reported that freezing lowered it. Changes in these and other milk components might, through their interfering effects for producing urea estimates by the IR method, be responsible for those changes in urea estimates that were observed in this study, in samples that were frozen for 4 or 7 d. However, that MU concentrations remained unchanged in preserved samples that were frozen for up to 3 d after collection indicates that occasional short-term freezing (i.e., overnight) of samples during transport to the laboratory should not interfere with the accuracy of the MU results produced by IR analysis.

Milk urea results did not differ between preserved samples that were stored at room temperature (21°C)

for up to 4 d, compared with samples that were refrigerated (4°C). These results agree with Miettinen and Juvonen (15) who reported that samples preserved with sodium azide could be stored at room temperature (20°C) for up to 7 d without affecting MU concentrations. These results indicate that the short-term storage of samples at moderate temperatures (21°C) in unrefrigerated courier trucks, during transport to the Ontario DHI laboratory, should not interfere with the quality of the MU results produced by IR analysis. This study did not examine the stability of MU concentrations in samples stored at high ambient temperatures (e.g., 30°C).

CONCLUSIONS

When interpreted at the group level, there was good overall agreement between MU results from the Fossomatic 4000 Milk Analyzer, an IR method, and the Eurochem test, an accepted reference method. The two tests shared a similar and high level of precision. Milk samples, as they are routinely collected and handled by the Ontario DHI field and laboratory staff, are suitable for analysis of MU concentrations by IR methods of analysis, provided that data are interpreted at the group level.

ACKNOWLEDGMENTS

This study was funded by a grant from the Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, ON. The authors gratefully acknowledge Louise O'Shaughnessy and the laboratory staff of the Ontario Dairy Herd Improvement Corporation for their technical support and their assistance with the transfer of necessary data. We also thank Eleanor Robinson, Janice Mitchell, Jeromy Tenhag, and the staff at the University of Guelph Ponsonby and Elora Dairy Research Stations for their willing assistance with sample collection.

REFERENCES

- 1 Baker, L. D., and J. D. Ferguson. 1994. Milk urea nitrogen as a metabolic indicator of protein feeding efficiency on dairy farms. *Bovine Pract.* 26:165-166.
- 2 Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. *J. Dairy Sci.* 78:2424-2434.
- 3 Barkema, H. W., J. Van der Schans, Y. H. Schukken, A.L.W. De Gee, T.J.G.M. Lam, and G. Benedictus. 1997. Effect of freezing on somatic cell count of quarter milk samples as determined by a fossomatic electronic cell counter. *J. Dairy Sci.* 80:422-426.
- 4 Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet* 1:307-310.
- 5 Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964-2971.

- 6 Burtis, C. A., and E. R. Ashwood. 1994. *Tietz - Textbook of Clinical Chemistry*. 2nd ed. W. B. Saunders Co., Toronto, ON.
- 7 Cannas, A., A. Pes, R. Mancuso, B. Voderet, and A. Nudda. 1998. Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes. *J. Dairy Sci.* 81:499-508.
- 8 Carlsson, B. J., and J. Bergstrom. 1994. The diurnal variation of urea in cow's milk and how milk fat content, storage and preservation affects analysis by a flow injection technique. *Acta Vet. Scand.* 35:67-77.
- 9 Gonda, H. L., and J. E. Lindberg. 1994. Evaluation of dietary nitrogen utilization in dairy cows based on urea concentrations in blood, urine and milk, and on urinary concentration of purine derivatives. *Acta Agric Scand. Sect. A. Anim. Sci.* 44:236-245.
- 10 Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. *J. Dairy Sci.* 76:475-484.
- 11 Kolver, E. S., and K. L. MacMillan. 1993. Short term changes in selected metabolites in pasture fed dairy cows during peak lactation. *Proc. N.Z. Soc. Anim. Prod.* 53:77-81.
- 12 Lee, A. J., A. R. Twardock, R. H. Bubar, J. E. Hall, and C. L. Davis. 1978. Blood metabolic profiles: their use and relation to nutritional status of dairy cows. *J. Dairy Sci.* 61:1652-1670.
- 13 Lee, K. L., K. P. Dayton, G. Kroll, and M. L. McGilliard. 1986. Effects of preservative, storage time, and storage temperature on milkfat percent, protein percent and somatic cell count determination. *J. Dairy Sci.* 69(Suppl. 1):211.(Abstr.)
- 14 Lefler, D. 1997. Comparison of the analytical characteristics of the enzymatic methods for urea determination in milk. *Station de Recherches en Technologic et Analyses Laitieres. Poligny, France.*
- 15 Miettinen, P. V., and R. O. Juvonen. 1990. Diurnal variation of serum and milk urea concentrations in dairy cows. *Acta Agric. Scand.* 40:289-296.
- 16 Oltner, R., M. Emanuelson, and H. Wiktorsson. 1985. Urea concentration in cows milk in relation to milk yield, live weight, lactation number and composition of feed given. *Livest. Prod. Sci.* 12:45-57.
- 17 Oltner, R., and L. Sjaunja. 1982. Evaluation of a rapid method for the determination of urea in cow's milk. *Acta Vet. Scand.* 23:39-45.
- 18 Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livest. Prod. Sci.* 10:457.
- 19 Payne, J. M., G. J. Rowlands, R. Manston, and S. M. Dew. 1973. A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. *Br. Vet. J.* 129:370-381.
- 20 SAS Institute Inc. 1992. SAS technical report P-229. SAS/STAT Software: Changes and enhancements, release 6.07. Cary, NC.
- 21 Schepers, A. J., and R. G. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. *J. Dairy Sci.* 81:579-584.
- 22 Shoukri, M. M., and V. L. Edge. 1996. *Statistical Methods for Health Sciences*. 1st ed. CRC Press, Inc., Boca Raton, FL.
- 23 Shoukri, M. M., and C. Pause. 1998. *Statistical Methods for Health Sciences*. 2nd ed. CRC Press, Inc., Boca Raton, FL.
- 24 Steel, R.G.D., and J. H. Torrie. 1980. *Principles and Procedures of Statistics: a Biometrical Approach*. 2nd ed. McGraw-Hill, Inc. New York, NY.
- 25 Timms, L. L., J. Connelly, and B. Dokkebakken. 1987. Effects of different containers, thaw methods, and preservatives on DHI fat, protein, and somatic cell measurements. *J. Dairy Sci.* 50(Suppl. 1): 135.(Abstr.)
- 26 Tyrrell, H. C., and P. W. Moe. 1975. Effect of intake on digestive efficiency. *J. Dairy Sci.* 58:1151-1163.