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EVALUATION OF A RAPID METHOD FOR THE DETERMINATION OF UREA IN COW'S MILK

By

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OLTNER, R. and L.-O. SJAUNJA: *Evaluation of a rapid method for the determination of urea in cow's milk.* Acta vet. scand. 1982, *23*, 39—45. — A rapid method for the determination of urea in milk was evaluated. The method employed was a urease/glutamate dehydrogenase procedure where the decrease in absorbance of NADH is monitored spectrophotometrically at 340 nm. The repeatability expressed as a coefficient of variation, was 1.4 % and 0.8 % for whole milk and skim milk, respectively. The accuracy of the method for urea determinations in whole milk was satisfactory (coefficient of variation, 2.6 %). Adding the preservative bronopol (0.02 %) to the milk or freezing of the milk samples for one week did not alter urea levels significantly ($P > 0.05$). The storage of preserved whole milk in a refrigerator for 14 days, however, increased milk urea levels measured by 3.1 % ($P < 0.001$).

It was concluded that the method evaluated is simple, rapid (40—50 samples/h) and yields reliable results also when whole milk is used. Since the determination of milk urea may be useful in assessing the feeding of energy and protein, the method is suitable for integration in the existing milk recording system in Sweden.

urea determination; milk; cows.

Blood concentrations of urea in ruminants are readily affected by dietary factors, especially the amount of protein ingested and the protein-energy ratio (*Lewis* 1957, *Ide et al.* 1966, *Waldo* 1968). Several reports have also demonstrated a close correlation between urea levels in blood and milk (*Peskett* 1934, *Ide et al.* 1966, *Journet et al.* 1975, *Eckart* 1980).

Since milk is so easily obtainable it is a highly suitable medium for the analysis of urea. So far, however, even recently applied methods for the analysis of urea in milk have been

rather time consuming (e.g. *Eckart* 1980, *Wolfschoon-Pombo et al.* 1981), which limits their applicability for extended routine analysis.

In this study we have evaluated a rapid and simple procedure for milk urea determinations permitting 1 person to analyse 40—50 samples per hour.

MATERIALS AND METHODS

Milk samples

Samples of composite milk were taken from 20 dairy cows of different breeds (mainly Swedish Red and White and Swedish Friesian). After thorough mixing of the samples they were divided into aliquots for the various treatments.

Experimental design

The repeatability of the method was estimated for whole milk and for the corresponding skim milk. The skim milk was obtained after centrifugation and subsequent removal of milk fat. Repeatability was estimated using the absolute difference (d_i) between duplicate determinations of 20 samples (n) and expressed as standard deviation ($s = \sqrt{(\Sigma d_i^2)/2n}$) and coefficient of variation (C.V., %).

The accuracy of the method was determined by adding known amounts of urea to the milk before analysis. Before estimating the regression of measured urea concentration (Y) on added concentration (X), the endogenous level of urea in the milk used was subtracted. The residual standard deviation (S_e) of the regression expressed the accuracy of the method.

The possible effect of the preservative bronopol (2-bromo-2-nitropropane-1,3-diol) on the milk urea determinations was investigated by paired comparisons of urea levels in whole milk without and with 0.02 % (w/w) bronopol.

The effect of freezing the milk samples on the measurement of urea was examined by paired comparisons of results obtained before and after 7 days in a freezer (—18°C). Prior to freezing, the milk samples were gently shaken to prevent separation of the milk fat.

To evaluate the effect of storage time on the urea levels, paired comparisons were made between fresh milk samples and preserved samples stored for 14 days in a refrigerator (+4°C).

Method of analysis

Urea in the milk was analysed using an I.L. 919 glucose/urea/creatinine analyser (Instrumentation Laboratories, Milano, Italy). The equipment is designed mainly for the determination of glucose, urea and creatinine in blood plasma or serum. According to the manufacturer, however, other body fluids as well as non-biological samples can also be assayed, provided that the concentration of the constituents is within the display range of the instrument.

Briefly, urea is analysed by the urease/glutamate dehydrogenase procedure. Urea is hydrolysed by the enzyme urease to give ammonium carbonate:

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3$$

The ammonium ions formed then act as a rate-limiting substrate for the glutamate dehydrogenase (GLDH) reaction:

$$\alpha\text{—ketoglutarate} + NH_4^+ + NADH \rightarrow NAD^+ + \text{glutamate} + H_2O$$

The decrease in absorbance at 340 nm following the oxidation of NADH is monitored spectrophotometrically over a period of 16 sec.

RESULTS

For the 20 cows used, the mean level of urea ($\pm$ s) was 5.82 $\pm$ 0.38 mmol/l in the whole milk and 6.21 $\pm$ 0.46 mmol/l in the skim milk. The standard deviation of the difference between duplicate determinations was 0.08 mmol/l for whole milk and 0.05 mmol/l for skim milk, yielding coefficients of variation of 1.4 % and 0.8 %, respectively. Duplicate determinations of urea in skim milk varied thus within $\pm$ 0.10 mmol/l as compared with $\pm$ 0.16 mmol/l for the whole milk samples ($P < 0.05$).

In Fig. 1, the accuracy of the method using whole milk is presented. The regression line between measured and expected urea concentrations was estimated to $Y = 1.02\ X - 0.22$. The residual standard deviation (S_e) of the regression was 0.21 and the coefficient of variation 2.6 %.

Urea levels in the fresh whole milk samples were not affected by addition of preservative (0.02 % bronopol), or freezing ($P > 0.05$). The differences between the means of the 20 samples without and with bronopol and before and after freezing were 0.03 mmol/l and 0.01 mmol/l, respectively.

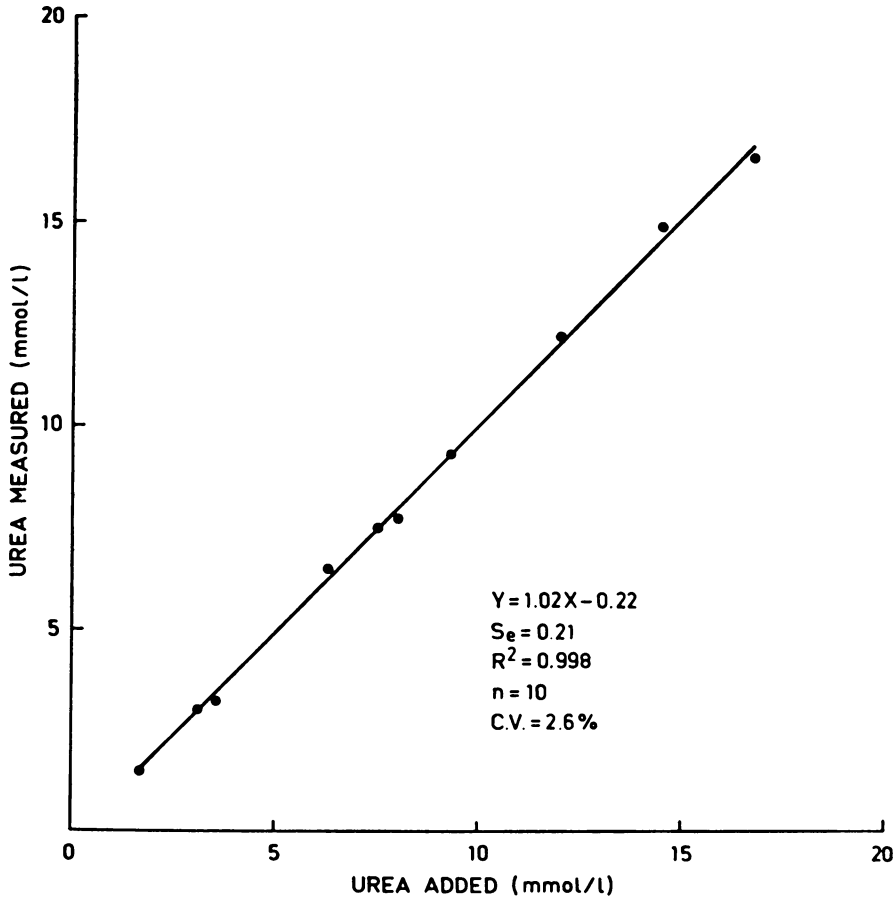

Figure 1. Accuracy of the method employed shown as the regression between milk urea concentrations measured (Y) and amounts of urea added to the milk (X).

When preserved milk samples ($n = 20$) were stored in a refrigerator for 14 days, however, a slight but significant increase in the mean urea concentration was observed (0.18 mmol/l; $P < 0.001$).

DISCUSSION

Levels of urea in milk from cows fed varying amounts of energy and protein have been shown to vary within a rather broad spectrum (*Erbersdobler et al.* 1979). Particularly in relation to the biological variation the repeatabilities obtained here

for urea determinations in both whole milk and skim milk are quite satisfactory. The lower repeatability estimate obtained for whole milk as compared with skim milk is probably due mainly to difficulties in obtaining perfectly mixed whole milk samples. The fat probably also has a slight rate-limiting effect on the enzymatic reactions, thus explaining the greater than expected difference in urea levels between the skim milk and the whole milk (0.39 mmol/l). On the basis of a pure dilution effect from the fat a difference of approximately 0.3 mmol/l would have been expected (mean fat percentage, 4.5). Nevertheless, repeatabilities of urea determinations in whole milk are quite sufficient for practical purposes.

The accuracy of the method as estimated from the analysis of whole milk with various amounts of urea added was also satisfactory. The coefficient of variation (2.6 %), is similar to that reported for the determination of other milk constituents such as fat and protein in milk from individual cows using infrared instruments (*Grappin & Jeunet* 1976).

Preservation with bronopol or freezing and thawing of the whole milk samples did not affect urea levels measured ($P>0.05$). Storage of preserved samples in a refrigerator for 14 days, however, increased the urea levels slightly (0.18 mmol/l, $P < 0.001$). Since ammonium ions act as a rate-limiting substrate in the reaction employed, ammonia already present in the samples will cause overestimation. It is known that because of the activities of proteolytic bacteria, ammonia may form in stored milk. Most likely this explains the observed increase in urea levels. Even in fresh milk, however, low and fairly constant levels of ammonia are found (*Venkatappaiah & Basu* 1952). When using the present assay technique, this presence will probably lead to a slight, systematic overestimation (< 0.2 mmol/l). From a practical point of view, however, the possible overestimation must be considered as negligible.

It can be concluded that the method evaluated here is accurate, rapid and convenient for the analysis of urea in milk. No pretreatment of the whole milk is required, which enables 1 person to analyse 40—50 samples per hour. The overall practicability of the method thus makes it suitable for use on a large scale for the charting of milk urea variations in relation to feeding of dairy cows.

REFERENCES

Eckart, K.: Bestimmung des Harnstoffsgehalts in der Milch. — Ein Beitrag zur Beurteilung der Protein- und Energieversorgung bei Kühen. (Milk urea level as an indicator of protein and energy intake of dairy cows). Thesis, Munich 1980.

Erbersdobler, H. F., K. Eckart & H. Zücker: Harnstoffanalysen in der Milch unterschiedlich versogter Kühe. (Milk urea concentrations in dairy cows with different protein and energy consumption). Landw. Forch., Sonderh. 36, Kongressband, Giezzen 1979, p. 98—103.

Grappin, R. & R. Jeunet: Essais de l'appareil Milko-Scan 300 utilisé pour le dosage en serié de la matière grasse et des proteines du lait. (Tests on the Milko-Scan 300 used for routine determination of fat and proteins in milk). Le Lait 1976, *56*, 498—520.

Ide, Y., K. Shimbayashi & T. Yonemura: Effect of dietary conditions upon serum- and milk-urea nitrogen in cows. I. Serum- and milk-urea nitrogen as affected by protein intake. Jap. J. vet. Sci. 1966, *28*, 321—327.

Journet, M., R. Verite & B. Vignon: L'azote non protéique du lait: facteurs de variation. (Non-protein nitrogen in milk: factors of variation). Le Lait 1975, *55*, 212—223.

Lewis, D.: Blood-urea concentration in relation to protein utilization in the ruminant. J. agr. Sci. (Camb.) 1957, *48*, 438—446.

Peskett, G. L.: Milk secretion in relation to blood composition. The urea contents of blood and of milk-serum. Biochem. J. 1934, *28*, 1657—1658.

Venkatappaiah, D. & K. P. Basu: Non-protein nitrogenous constituents of milk. Indian J. Dairy Sci. 1952, *5*, 95—116.

Waldo, D. R.: Symposium; Nitrogen utilization by the ruminant. Nitrogen metabolism in the ruminant. J. Dairy Sci. 1968, *51*, 265—275.

Wolfschoon-Pombo, A., H. Klostermeyer, J. Buchberger & R. Graml: Harnstoff in der NPN-fraktion der Kuhmilch — Bestimmung, Vorkommen und Beeinflussung. (Urea in the NPN-fraction of cow's milk — determination, content and influence on it). Milchwissenschaft 1981, *36*, 462—466.

SAMMANFATTNING

Utvärdering av en snabb metod för bestämning av urea i komjölk.

Metoden bygger på en ureas/glutamatdehydrogenas reaktion där i slutsteget oxideringen av NADH ger en absorbansminskning som avläses spektrofotometriskt vid 340 nm. Reproducerbarheten, uttryckt som variationskoefficient, för ureabestämning i helmjölk och skummjölk var 1.4 % respektive 0.8 %. Genom tillsats av kända mängder urea till helmjölk före analys kunde konstateras en mycket god överensstämmelse mellan erhållna och förväntade värden (variationskoefficient 2.6 %). Bronopol i koncentrationen 0.02 % påverkade inte

mjölkureabestämningen ($P > 0.05$). Inte heller frysning av helmjölken under en vecka hade någon påvisbar effekt ($P > 0.05$). Efter lagring av helmjölk med bronopoltillsats i kylskåp under två veckor, kunde däremot konstateras en ökning av mjölkurea med i genomsnitt 3.1 % ($P < 0.001$).

Slutsatsen blev att den evaluerade metoden är enkel och snabb, (40—50 prov per timme), samt ger pålitliga resultat även vid analys av urea i helmjölk. Då analys av mjölkurea kan ge en uppfattning om huruvida utfodringen av energi och protein är korrekt, torde en integrering i den befintliga kokontrollens analyssystem vara av intresse.

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