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Analysis of Milk Urea Nitrogen by an Inexpensive Near Infrared Spectral Analysis at Wavelengths Less than 1100nm

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Urea nitrogen concentration in milk (MUN) is known an indicator of protein-energy balance in animals. Information of MUN is important and should be continuously measured in dairy farms. Near infrared spectroscopy (NIRS) is a promising technology for routine and daily measurement because it is a fast, accurate and non-pollutant method. Our previous study on the use of NIRS for predicting MUN content from milk liquid samples failed (Purnomoadi *et al.*, 1999a), but an alternate study using urine samples (Purnomoadi *et al.*, 1999b) showed that absorbance of urine in NIR spectra was highly correlated with urea concentration in blood. However, that study was done on a wavelength range of 1100-2500 nm. To develop inexpensive analytical equipment, a study on wavelengths shorter than 1100 nm was carried out.

Two hundred and thiry paired samples of milk and urine was collected from eight multiparous Holstein cows in midlactation. Those cows were fed Italian ryegrass hay, beetpulp and commercial concentrate to meet maintenance and milk production requirements. Four cows were allowed 13%CP (CP13), two cows were supplemented with soybean meal to make 18%CP (SB18) and two cows were fed fishmeal to make 18%CP (FM18), respectively. Milk samples were collected in the morning and evening. Spot urine samples were collected by vulval stimulation thirty minutes before milking. The samples were stored at -35C until chemically analysed. Urea N concentration in milk was analysed with the Urease Indophenol method using commercial kit (Wako Pure Chemical Ltd., Japan).

Near infrared spectra of urine samples were scanned with a Pacific Scientific (Neotec) model 6500 instrument (Perstorp Analytical, MD) using a transmittance cell sample (thickness: 1mm). Spectra were read at 2 nm intervals over the range of wavelength between 400 and 1100 nm. The spectra were then calculated as the second derivative of log[1/A]; where A is the absorbance, using ISI software (InfraSoft International, Port Matilda, PA). The calibration equation was developed by step-wise linear regression using maximum four-wavelength combination. For NIR spectroscopy analysis, urine samples were separated into two groups. The first group which was a set of samples (n=112) for developing the calibration equation, was prepared from four cows (two each of CP13 and SB18). The MUN in this group ranged between 7.7-30.2 (13.9) mg/dl. The second group provided the samples (n=118), were used for validating calibration equation. This group comprised the rest of each two cows of CP13 (n=78, MUN range 8.2-23.2 (12.7) mg/dl) and FM18 (n=40, 14.9-32.3 (20.0) mg/dl).

The results showed that a wavelength around 1000 nm was highly correlated with urea concentration in milk (r=0.81). The strength of that correlation varied between animals and seems to be influenced by feeding regimes. In this study a combination of that wavelength (1000 nm) and 926 nm was the most suitable calibration equation for predicting MUN with respect to the reproducibility and accuracy of measurement.

Application of that calibration equation on the validation samples resulted in a standard error of prediction as of 1.5 mg/dl (with r=0.77) for CP13 cows, and 1.7 mg/dl (r=0.42) for FM18 cows. The lower accuracy of prediction in FM18 than in CP13 maybe due to (1) the FM18 was not included in calibration samples and (2) its distribution of MUN values was different from the calibration. The finding of wavelengths shorter than 1100 nm available for estimating MUN in this study is a promising inexpensive tool for monitoring the nutritional sufficiency through urine analysis. The accuracy of calibration equation can be improved by the inclusion of the representative spectra under different feeding regimes. Further studies to improve the accuracy through enlarging the absorbance in NIRS spectra may also be carried out using a cell sample thicker than 1mm.

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